THE ECOLOGY OF VAM FUNGI IN CONTRASTING AUSTRALIAN AGRICULTURAL SYSTEMS

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Declaration

The research presented in this thesis is my original and independent work. Specific contributions and assistance by others are referred to in the text and acknowledgements.

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Abstract

While much is known about the functioning of vesicular-arbuscular mycorrhizal (VAM) fungi under controlled conditions, relatively little research has been conducted into the influence of VAM fungi on ecosystem functioning. In particular, there has been little attempt to link what is known about the functioning of VAM fungi at the scale of the individual plant with broader ecological processes. In part, this deficiency results from the difficulties inherent in conducting research into VAM fungi under field conditions.

This thesis presents the results of a field-orientated project that examined the ecology of VAM fungi in agricultural systems in SE Australia between 1993 and 1996. Ecological trends involving VAM fungi, phosphorus (P) and plant growth were investigated using 23 farm pairs consisting of adjacent commercial farms under contrasting farm management strategies; conventional and alternative. The conventional farmers applied large inputs of soluble P fertilisers, while the alternative farmers either applied no P fertilisers or applied the relatively insoluble rock phosphate. This design allowed the long term effects of variations P inputs to be examined on replicated sites. The paired farms were located in two contrasting agricultural commodity production systems; a dryland (mixed) cereal-livestock system which alternated between annual pasture and annual crops, and a dairy system which consisted of permanent, perennial, irrigated pasture. Farms of the same commodity production system and farm management strategy were regarded as replicates. The project was designed to test hypotheses about the ecology of VAM fungi at both the scale of the VAM fungi-plant relationship and, more broadly, at the scale of ecosystem-level processes. A major aim was also to test the hypothesis that the biological processes in conventional and alternative agricultural systems are fundamentally different.

For both wheat crops and subterranean clover in pastures on the mixed farms, and the three major species (rye grass, paspalum and white clover) in the pastures on the dairy farms, VAM colonisation levels were consistently strongly negatively correlated with soil extractable P and plant P concentrations. The negative influence of P was confirmed in glasshouse trials. Total soil N and plant N concentrations were positively correlated with VAM colonisation in some instances. On the mixed farms, a severe drought markedly reduced VAM colonisation in annual crops in 1994 and appeared to reduce the VAM inoculum potential of the soil. On individual dairy farms, VAM colonisation levels in pasture did not vary greatly over a four year period.

Glasshouse trials were used to assess the contribution of VAM fungi to plant growth in soil taken from mixed farms and dairy farms. The glasshouse trials were designed to be as relevant to field conditions as possible. They also assessed the effects of factors that may often vary between glasshouse and field conditions, including plant density (intraspecific competition), interspecific competition, defoliation (grazing) and the

species of VAM fungi. In spite of low soil extractable P, wheat grown in soil from the mixed farms received no yield benefits from VAM colonisation, however growth of subterranean clover was substantially increased by VAM colonisation. Rye grass and paspalum grown in soil from the dairy farms received little net growth increase from VAM colonisation, while white clover received a small net benefit. Compared to the mixed farm soil, the higher soil extractable P in the dairy farm soil reduced the benefits from VAM colonisation for clover growth. High plant densities reduced the response to VAM colonisation of white clover; interspecific competition, defoliation and variation in VAM species had no effect.

Due to the addition of fertilisers containing soluble P, conventional farms consistently had higher soil extractable P concentrations and significantly lower VAM colonisation levels than alternative farms. No other farm management practices specific to conventional or alternative management were found to significantly affect VAM colonisation levels. Thus, VAM fungi made a greater contribution towards nutrient uptake on the alternative farms, however, in terms of yield, this did not compensate for the non-application of soluble P fertilisers. However, VAM fungi probably contributed towards other processes on the alternative farms, including closing of nutrient cycles, pathogen control and soil structure maintenance. Alternative farms also had lower levels of *Rhizobium* nodulation in clover than conventional farms, probably due to lower plant P concentrations limiting formation of nodules.

In spite of greater than 30 years of differing fertiliser applications, there was no indication that the VAM community differed between conventional and biodynamic dairy farms. VAM spore types and abundance did not differ significantly between the farms and, in a glasshouse trial, VAM colonisation and plant growth in conventional and biodynamic dairy farm soils responded in the same manner to nutrient additions. It was concluded that the soil biological interactions governing processes such as plant nutrient uptake are similar on conventional and alternative farms, but vary along a continuum — in a predictable manner — in response to quantifiable factors, such as inputs of P. There was no indication that alternative farms function through processes not able to be defined using current scientific methods and knowledge, as has been claimed by some people. Overall, P inputs and the levels of VAM fungi may be the most consistent, significant difference between the soil ecosystems on conventional and alternative farms in Australia.

Farmers in these agricultural systems do not need to change their management practices to favour VAM fungi. Alternative farms will have close to maximum levels of VAM colonisation in crops and pastures and only a major event, such as severe drought, will reduce VAM levels in crops. However, as no VAM dependent crops are grown, or seem likely to be grown, in the SE Australian wheatbelt, this will not affect crop yields. Conventional farms would have to forgo the benefits for yields from applying soluble P fertiliser to increase VAM colonisation in crops or pasture. However, the high

colonisation in the mixed farm pastures and the relatively high colonisation in the dairy pastures means that other benefits from VAM colonisation, such as improved soil structure, will be present on conventional farms. These other functions of VAM fungi may provide VAM fungi with an important role in the development of more sustainable agricultural systems.

Regarding the general functioning of VAM fungi, it was concluded that the costs and benefits to plants from VAM colonisation are often closely balanced, but as regulation of VAM fungi by the plant is relatively loose, minor negative balances for plants can occur. This occurred in a glasshouse trial due to low light levels. On a broad scale, VAM fungi may act to buffer plant P concentrations as soil P decreases. Due to variation in the scale at which trends involving VAM fungi become clear, results from field surveys must be supported by field or glasshouse manipulative experiments to confirm which variables are responsible for the trends noted in the field.

Glossary

Available P: the portion of P in the soil which can be absorbed by plant roots.

Alternative Agriculture: farm management strategies which conform to the Australian National Standard for Organic and Biodynamic Produce; these include organic and biodynamic agriculture.

Annual Pasture: pasture that grows on an annual cycle; senescent during summer.

Anthesis: stage in the lifecycle of an annual cereal crop when flowering begins.

Biodynamic (BD): alternative farm management based on the teachings of the Austrian philosopher Rudolf Steiner.

Check Bank: low contour bank around 50-100 mm high and 0.5 m wide used in flood-irrigated pasture paddocks to aid with even water distribution.

Conventional Agriculture (Con.): the farm management strategy used by the majority of Australian farmers which includes use of pesticides and fertilisers containing water soluble nutrients.

Deficient Nutrient Concentration: nutrient concentration in plant material which is too low for optimum performance; visible symptoms are present.

Direct Drilling: sowing seed into slots or holes in the ground without first cultivating the ground.

Extractable P: The portion of P in the soil which can be easily extracted in the laboratory by tests designed to mimic the capacity of plant roots to absorb P, however it is not necessarily the same as available P.

Facultatively Mycotrophic: Plants which can survive with or without mycorrhizal colonisation.

Fallow: soil kept free of plant growth by tillage or herbicides to conserve moisture for a period prior to the sowing of a crop.

Fallow Crop: first crop after a paddock has been under fallow, generally following a pasture phase.

First Year Crop: first crop after a paddock has been in a pasture phase.

Growing Season: the period from when the crop was sown until senescence, usually May/June to November for winter crops in SE Australia.

Laser Levelling: use of laser technology to survey a paddock while levelling the paddock to give a constant slight slope and allow removal of check banks.

Leaf P or N: concentration of P or N in the youngest 2-3 fully-emerged leaves.

Low Nutrient Concentration: no symptoms, but nutrient concentration in plant material may be too low for optimum plant performance.

Minimum Tillage: Soil disturbance is the minimum necessary to place the seed in a favourable environment and all plant residue is left on the soil surface.

Mycotrophic: Plants which require colonisation by mycorrhizal fungi for survival.

N: used to denote nitrogen in any molecular form.

Nonmycotrophic: plants which are never colonised by mycorrhizal fungi.

Normal Nutrient Concentration: no symptoms, concentration in plant material is adequate for plant growth.

Organic: alternative farm management other than biodynamic.

P: used to denote phosphorus in any molecular form.

Pasture P or N: concentration of P or N in pasture; all species combined.

Pasture Phase: a paddock is returned to pasture for a number of years before being used for cropping.

Perennial Pasture: pasture that does not senescence over summer and therefore contains perennial plants.

Plant Growth Potential: the degree of plant growth in a particular soil in a glasshouse trial after a designated period of time.

Root P or N: concentration of P or N in roots.

Second Year Crop: second crop planted after a paddock has been in a pasture phase.

Seed Dressing: coating seed with fungicide before sowing; 'pickling'.

Shoot P or N: concentration of P or N in shoot material.

Shoot P (or N) Content: weight of P of N in shoots in a designated area of pasture or crop.

Soil Mat: mat of organic material on the soil surface comprising plant litter and roots in varying degrees of decomposition.

Stubble: the crop material left in a paddock after the grain has been harvested.

Summer Crop: crop grown over summer; December to April. Can only be grown in areas of the wheatbelt which receive adequate summer rainfall.

Tillering: stage in the lifecycle of an annual cereal crop when tillers (basal branches arising from buds in the axils of the leaves on the stem) begin to form.

Tillage: cultivation, ploughing.

Traditional Tillage: soil is inverted and/or broken-up and left bare.

VAM (%): the percentage of root length colonised by VAM fungi.

VAM Intensity: the percentage of root length colonised by VAM fungi adjusted for the intensity of the colonisation.

VAM Fungi: vesicular arbuscular mycorrhizal fungi.

VAM Inoculum Potential: the degree of VAM colonisation in a particular soil cause in a glasshouse trial after a designated period of time.

Winter Crop: crop grown over winter; May/June to November.

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Part A

General Introduction

This thesis presents the results of a field-orientated project that examined the ecology of vesicular-arbuscular mycorrhizal (VAM) fungi in contrasting agricultural systems in SE Australia. The project was designed to test hypotheses about the ecology of VAM fungi — an area for which knowledge is currently limited — at both the scale of the VAM fungi-plant relationship and, more broadly, at the scale of ecosystem-level processes. A major aim was also to test the hypothesis that the biological processes in conventional and alternative agricultural systems are fundamentally different. Part A contains two chapters: Chapter 1 provides an introduction to VAM fungi and a review of their ecology, before the specific project aims are presented; Chapter 2 consists of background information on the agricultural systems which were investigated.

Chapter One

Vesicular-Arbuscular Mycorrhizal Fungi

This chapter introduces the organisms which are the primary focus of this thesis; vesicular arbuscular mycorrhizal fungi. The lifecycle of VAM fungi is described, followed by a review of their interactions with host plants, particularly in regard to phosphorus (P) nutrition. Their role in agricultural systems is then described and the difficulties with assessing the functioning of VAM fungi under field conditions considered. The chapter concludes with a brief discussion of the role of VAM fungi in broad-scale ecological processes and the presentation of the specific project aims.

1.1 Introduction

A mycorrhiza is a symbiotic association between a plant root and a fungus in which energy moves primarily from plant to fungus in the form of photosynthates, while inorganic resources from the soil are transferred from the fungus to the plant (Allen 1991). Mycorrhizas can be divided into morphological groups based on the degree to which the fungus penetrates the host plant root (Allen 1991). In ectomycorrhizas, which occur on many forest trees, the fungus does not enter plant cells, but forms a tissue surrounding the root (mantle) and interpenetrating between epidermal cells (Hartig net). In endomycorrhizas there is no mantle, instead, fungal hyphae invade cells of the root cortex and the plasma membrane grows to surround the invading hyphae, but is not breached. Thus the fungus remains outside the plant cell cytoplasm (Smith and Read 1997).

1.2. The Nature of Vesicular-Arbuscular Mycorrhizas

The most commonly formed endomycorrhizas are vesicular-arbuscular mycorrhizas (VAM) which are formed by fungi in the order Glomales (Zygomycotina). These are named after two distinctive specialised structures, arbuscules and vesicles, which are formed within the plant cortex cells (Fig. 1.1). Arbuscules, which are characteristic of all VAM fungi, consist of branching clusters of fine hyphae and are thought to be the main site for nutrient exchange; although this may also occur at the interface between the intercellular hyphae and the root cells (Smith and Read 1997). Vesicles, which are formed by the majority of VAM fungi, are swollen sections of hyphae which appear to be fungal storage organs.

VAM fungi produce a diffuse external network of hyphae in the soil, which is connected to internal hyphae in the cortex of the host root. The external hyphae absorb nutrients from the soil and these are transported into hyphae in the root and absorbed by the host plant. In return, the VAM fungi have access to carbon compounds photosynthesised by the host plant; the primary energy source for the fungi (Smith and Read 1997). VAM fungi are obligate symbionts and cannot grow or reproduce without a host plant. Up to 20% of host plant photosynthate is required for the formation, maintenance and functioning of mycorrhizal structures (Jakobsen and Rosendahl 1990; Peng *et al.* 1993).

VAM associations are formed by approximately 150 species of fungi from six genera. They reproduce using multinucleate asexual spores, many of which are unusually large; 10-1000 μm in diameter. Spore morphology is the current basis for VAM taxonomy, although DNA and molecular techniques are under development (Clapp *et al.* 1995; Hahn *et al.* 1994) and examination of the morphology of the fungal

structures inside the root may also be useful (Abbott and Robson 1978; Abbott and Robson 1979). Pieces of external hyphae and dead roots containing hyphae and vesicles may also act as propagules. Existing colonies may spread through root systems by producing external hyphae which grow along, enter, and colonise new sections of root. The asexual reproduction of VAM fungi means that the described species cannot be regarded as either biological or ecological species, but rather as phenetic or form species (Smith and Read 1997) as they are functionally, and possibly genetically, diverse. Thus the characteristics of a VAM fungus varies with both the species and the isolate of the fungus (Abbott and Robson 1985b; Allen and Boosalis 1983; Gavito and Varela 1995; Graham *et al.* 1982).

VAM fungi associate with members of virtually all plant families, although a few families are predominantly non-mycorrhizal including Cyperaceae, Proteaceae, Brassicaceae, Polygonaceae and Chenopodiaceae (Brundrett 1991; Gerdemann 1968; Newman and Reddell 1987). There is no clear evidence that any absolute specificity exists between taxa of VAM fungi and taxa of host plants, although there is evidence that host plants may be preferentially colonised by particular VAM species (McGonigle and Fitter 1990). Thus, a number of species will generally be present in the soil at any location (Hayman and Stovold 1979) and even within an individual plant or section of root, different species often occur together (Wilson 1984; personal observation).

1.3. Functioning of VAM

1.3.a. VAM Fungi and Phosphorus

In the majority of instances where VAM colonisation has been found to lead to significant increases in host plant growth, the effect has been due to the fungi increasing plant P uptake through increased efficiency of P uptake by mycorrhizal roots (for example, Marschner and Dell 1994; Stribley *et al.* 1980; Thompson 1990). Phosphorus is required by plants in relatively large amounts, but often occurs in the soil at low concentrations and in insoluble forms not accessible to plants. In addition, P ions diffuse slowly through soil and depletion zones quickly form around roots. VAM hyphae can extend into the soil beyond depletion zones, absorb P and transport it to the plant faster than P can move through the soil solution (Smith and Read 1997). The small diameter of the hyphae allows access to smaller soil pores than plant roots can access. Overall, the presence of VAM fungi substantially increases the volume of soil from which a host plant can access P.

There is some suggestion that VAM fungi may be able to access inorganic sources of P in soil that are not available to roots (Achtnich and Moaward 1986; Allen *et al.* 1992; Mosse *et al.* 1976b; Powell and Daniel 1978), however, convincing experimental evidence is still lacking (Bolan 1991). Evidence of VAM accessing P

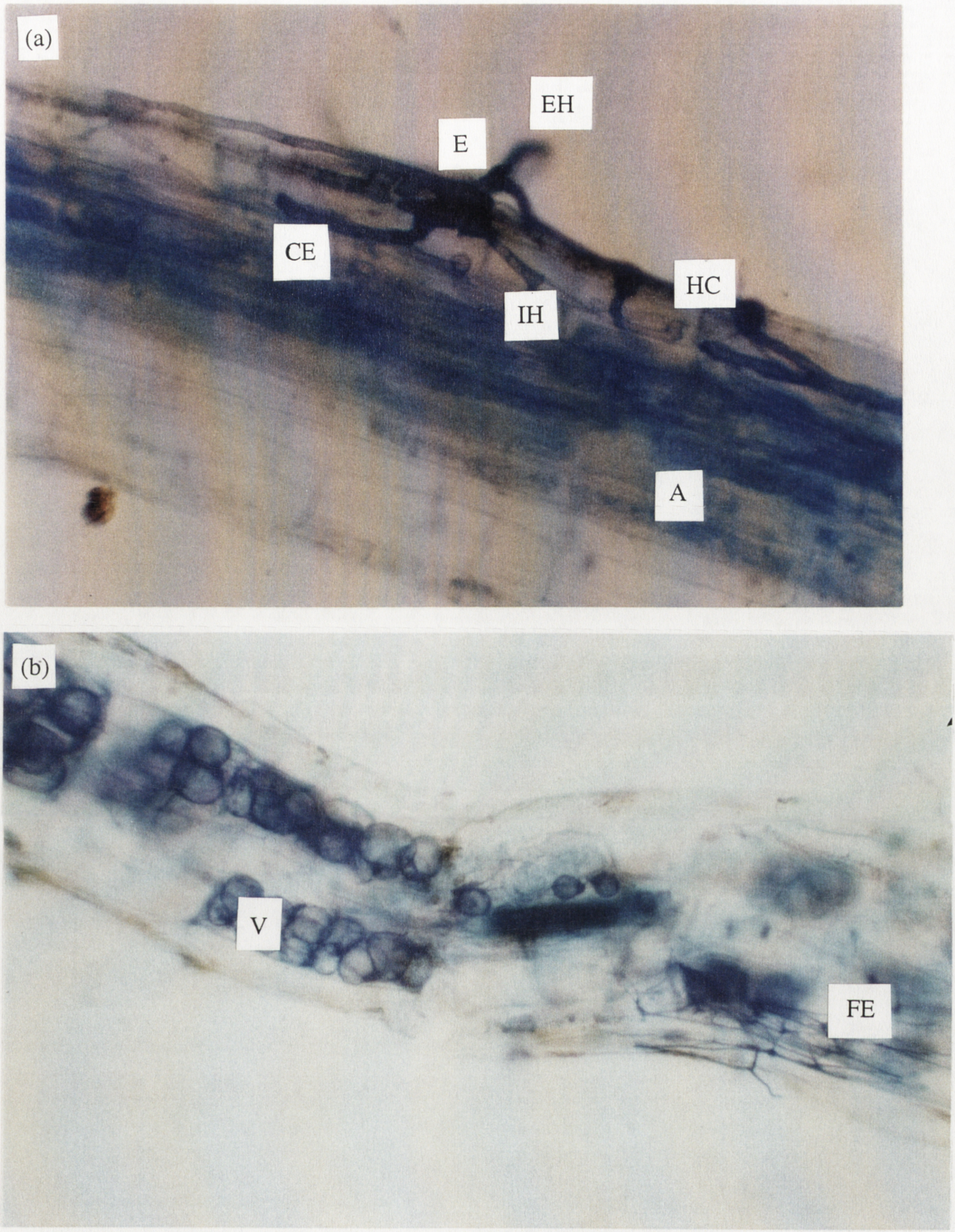


Figure 1.1. Wheat roots from an organic farm colonised by indigenous VAM fungi a) at tillering (x 150) and b) at anthesis (x 60). All fungal material has been stained blue and the following features are noted: external hyphae (EH); entry point (E); arbuscules (A); internal hyphae (IH); hyphal coils (HC) which are typical of colonisation by some species of VAM fungi; and vesicles (V). The younger root (a) is colonised by a species of VAM fungi which forms thick internal hyphae (coarse endophyte - CE) and the vesicles in the older root (b) are also likely to have been formed by this species. Most of the thick hyphae in the older root have broken down. The older root is also being colonised by a species of VAM fungi which forms thin hyphae (fine endophyte - FE).

from organic sources (Jayachandran and Schwab 1992; Tarafdar and Marschner 1994; Tarafdar and Marschner 1995), is likely to be due to hyphae allowing greater exploration of the soil, not to VAM fungi enhancing mineralisation (Joner and Jakobsen 1995).

When P is limiting plant growth, the presence of VAM fungi may enhance host plant growth (Abbott and Robson 1981; Buwalda *et al.* 1985a; Khan 1975). This may be evident as increased growth of roots and shoots, a reduced root-shoot ratio and increased plant P concentrations (Smith and Read 1997). A typical response of a host plant to colonisation by VAM fungi is shown in Figure 1.2. Clover (*Trifolium subterraneum* L.) was grown, in P-deficient soil, in a glasshouse trial under a number of levels of P, and with and without VAM inoculum. The presence of VAM fungi increased plant growth at low P levels, but this effect decreased as P levels increased (Bolan and Robson 1983). When the host plant has access to abundant P, VAM fungi may have a negative effect on growth, as the P they provide is not necessary for the plant to overcome a P deficiency, but the fungi still use photosynthate (Bethlenfalvay *et al.* 1982; Crush 1995; Gavito and Varela 1995; Olsen *et al.* 1996; Peng *et al.* 1993).

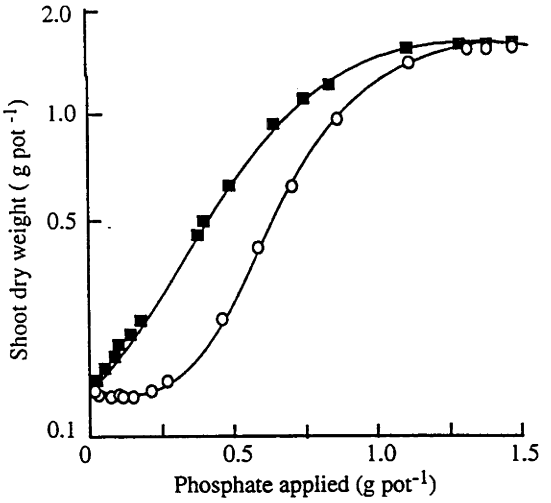


Figure 1.2. The effect of P application on the shoot dry weight of clover (*Trifolium subterraneum* L.) grown with and without VAM fungi. Redrawn from Bolan *et al.* (1983).

While VAM fungi may increase the concentration of P in the host plant, P may also control the extent of colonisation by VAM fungi. When soil P is low, additions of P may initially increase colonisation by VAM fungi (Bolan *et al.* 1984), but as more P is applied, the proportion of root length colonised and the number of spores produced generally decreases (Amijee *et al.* 1993b; Jasper *et al.* 1979; Mårtensson and Carlgren 1994; Raju *et al.* 1990). The degree that colonisation is depressed by P may also depend on the N status of the plant (Bååth and Spokes 1989); under N-deficient conditions, addition of P may not decrease colonisation levels (Sylvia and Neal 1990).

Also, any situation where the supply of photosynthate is reduced is likely to decrease VAM colonisation (Smith and Read 1997); in particular, low light levels and defoliation (Daft and El-Giahmi 1978; Smith and Gianinazzi-Pearson 1990). High P levels and low light levels have been found to interact, greatly reducing VAM colonisation (Son and Smith 1988). The exact mechanisms behind the effects of P, light and grazing on colonisation levels have not been elucidated. It has been suggested that the effect of P is mediated through the effects of the phospholipid content of root cells on root cell membrane permeability and exudation of the carbohydrates on which the fungi depend (Graham *et al.* 1981).

1.3.b. The Hyphal Network Concept

VAM hyphae have been shown in the laboratory to colonise and connect roots from plants of the same or a different species (Newman 1988). Furthermore, ^{14}C labelling experiments have indicated below-ground transfer of carbon between plant roots and movement of this carbon into shoots (Grime *et al.* 1987; Waters and Borowicz 1994). This has led to the concept of a 'hyphal network' linking mycorrhizal plants within a community and allowing exchange of nutrients (Martins 1992). Large net transfers of carbon or nutrients between plants have not yet been shown, however Smith and Read (1997) note that such a network could function through the sharing of nutrients acquired by the network, perhaps with some plants providing a greater share of the photosynthate needed to support the fungi.

It has also been suggested that a functional hyphal network may enhance the cycling of nutrients within a plant community and reduce nutrient leaching (Jeffries and Barea 1994). Internal VAM hyphae are well placed to absorb nutrients as plant roots decay and, if linked to a hyphal network, might transfer nutrients out of a dying plant directly to a linked living plant (Newman and Eason 1989). For instance, dying plants can lose up to 60% of P and N within three weeks after death, with much of this ending up in neighbouring plants; Newman and Eason (1989) found the magnitude of this transfer was substantially greater if the plants were mycorrhizal.

The occurrence and functional significance of hyphal networks under field conditions is still speculative. However, it is possible that — along with the ability of VAM fungi to affect competitive balance between host plants (Fitter 1977) — a functional hyphal network may allow VAM fungi to significantly influence the nature and composition of plant communities (Francis and Read 1994; Grime *et al.* 1987).

1.3.c. Other Influences of VAM Fungi on Host Plants and Ecosystem Functioning

In addition to enhancing plant P uptake, VAM fungi may directly influence the uptake by plants of other macro- and micro-nutrients (Marschner and Dell 1994), including zinc (Thompson 1990), copper (Wilson 1988a), nitrogen (Azcón *et al.* 1992; Barea *et al.* 1987) and potassium (Tarafdar and Marschner 1995).

There are some indications that VAM fungi may influence plant water relations (Allen and Boosalis 1983; Ellis *et al.* 1985; Nelsen and Safir 1982; Sánchez-Díaz and Honrubia 1994; Trent *et al.* 1989), although the mechanisms behind this effect have not yet been fully explained (see also §8.2.c.i). VAM fungi may also affect the interactions between plants and soil transmitted pathogens (Dehn 1982; Linderman 1992), both through improving the P nutrition of the host plant (Graham and Menge 1982) and through direct effects on the growth of the pathogen (Rosendahl and Rosendahl 1990; Thompson and Wildermuth 1989). Indeed, in natural systems the primary benefit from VAM fungi to the host plant may be protection against pathogens (Newsham *et al.* 1995; Watkinson *et al.* 1996). Through altering plant nutrient balances, VAM fungi may also affect insects (Gange and Nice 1997; Gange and West 1994).

VAM fungi also interact more broadly with their surrounding ecosystem. It is likely that the fungi are an important food source for many soil organisms (Brundrett 1991) and they have been shown to interact with other soil micro-organism populations (Daniels Hetrick *et al.* 1988; Linderman 1992; Meyer and Linderman 1986). VAM fungi also contribute towards the maintenance of soil structure through the contributions of both extracellular polysaccharides and external hyphae to the formation and stabilisation of soil aggregates (Bethlenfalvay and Barea 1994; Miller and Jastrow 1990; Miller and Jastrow 1992b; Tisdall 1991).

1.3.d. The Dependency of Host Plants on VAM Fungi

Plant species and genotypes differ in their growth response to VAM fungi (Manske 1989; Plenchette *et al.* 1983; Raju *et al.* 1990). The degree of growth response to VAM colonisation is termed 'mycorrhizal dependence' and it has been related to the morphology of the plant root system. Plants possessing fine roots with long root hairs, such as many grasses, often gain little benefit from VAM colonisation, while plants with relatively thicker roots and fewer root hairs, such as many legumes, generally exhibit a larger positive growth response to VAM colonisation (Schweiger *et al.* 1995). The high P requirement for nodulation in legumes may also increase dependency on VAM fungi (Kucey and Paul 1982; Mosse *et al.* 1976a; Vejsadová *et al.* 1989). Mycorrhizal dependency is also affected by many other factors, including the environmental conditions present, the extent of VAM colonisation and the rate of root growth (Smith and Read 1997).

1.4. VAM Fungi in Agricultural Systems

1.4.a. Agricultural Plants and VAM Fungi

VAM fungi are known to colonise many major crop and pasture species including wheat (*Triticum aestivum* L.) and barley (*Hordeum* spp.) (Jensen and Jakobsen 1980), rye grass (*Lolium* spp.) (Schweiger *et al.* 1995), sunflowers (*Helianthus annuus* L.) (Thompson 1987), linseed (*Linum usitatissimum* L.) (Thompson 1994a), peas (*Pisum* spp.) (Jakobsen and Neilson 1983), clover (*Trifolium* spp.) and medics (*Medicago* spp.) (Patterson *et al.* 1990). The effect of VAM fungi on crop yields varies from strongly positive (Thompson 1994a) to negative (Bethlenfalvay *et al.* 1982; Buwalda and Goh 1982; Hendrix 1985; Peng *et al.* 1993; see §1.3.d). There are some crops which are non-mycorrhizal including lupins (*Lupinus* spp.) and crucifers including cabbage (*Brassica oleracea* L.) and canola (*Brassica napus* L.) (Ocampo 1980; Ocampo *et al.* 1980; Thompson and Wildermuth 1989).

The main plant species examined in this project were white clover (*Trifolium repens* L.), subterranean clover (*T. subterraneum* L.), wheat (*Triticum aestivum* L.), perennial rye grass (*Lolium perenne* L.) and paspalum (*Paspalum dilatatum* Poir.). Clovers are generally highly dependent on VAM fungi (Puppi and Bras 1989; Schweiger *et al.* 1995). While VAM colonisation of wheat markedly increases growth in some instances (Khan 1975; Mohammad *et al.* 1995), this does not always occur (Buwalda *et al.* 1985a; Plenchette and Perrin 1992). Rye grass generally does not respond positively to colonisation by VAM fungi (Crush 1995; Schweiger *et al.* 1995); no information could be found on paspalum.

1.4.b. Effects of Agricultural Practices on VAM Fungi

As VAM fungi cannot grow without being associated with a host plant, culturing the fungi is difficult and, as yet, an economical large-scale method for inoculum production has not been developed. Thus, unlike other micro-organisms such as *Rhizobium*, use of VAM fungi in commercial scale agriculture generally involves manipulation of populations through farm management practices.

The effects of various farm management practices on VAM colonisation are summarised in Table 1.1. Effects of management practices, such as tillage regimes, may vary for different species of VAM fungi (Douds *et al.* 1995) and result in changed species composition (Johnson 1993). VAM colonisation levels will also be affected by environmental variables such as rainfall and season (Armstrong *et al.* 1992; Braunberger *et al.* 1994; Jasper *et al.* 1993; Scheltema *et al.* 1987; Smith 1980). Different cultivars of a species may differ greatly in their level of colonisation (Baon *et al.* 1993a).

Table 1.1. The influences of farm management practices used in the farming systems studied in this project on abundance of VAM spores, VAM colonisation levels or colonisation of subsequent crops (adapted from Smith and Read 1997, see also references listed at end of table).

Management Practice	Positive influence	Negative influence
Fertiliser application		
phosphorus	relatively insoluble	soluble
nitrogen	variable	
lime	changes VAM species composition	
Fungicides	variable	
Grazing	variable	
Herbicides	variable	
Plants grown	VAM-host species	non-VAM host species
	pasture	
	cover cropping	
Soil disturbance	minimum tillage	conventional tillage
		long fallow
		compaction
		soil erosion
Weeds, Irrigation, Stubble burning	unknown	

(Abbott and Robson 1985a; Baon *et al.* 1992a; Daft and El-Giahmi 1978; Evans and Miller 1990; Galvez *et al.* 1995; Jasper *et al.* 1989; Johnson 1993; Mårtensson and Carlgren 1994; Menge 1982; Mohammad *et al.* 1995; Nadian *et al.* 1996; Ocampo 1980; Ocampo and Barea 1985; Ocampo *et al.* 1980; Plenchette and Perrin 1992; Porter *et al.* 1987a; Porter *et al.* 1987b; Ryan *et al.* 1994; Smith 1978; Smith 1980; Sugavanam *et al.* 1994; Thompson 1987; Thompson 1994a; Thomson *et al.* 1992; Tommerup and Briggs 1981; Trent *et al.* 1988; Wallace 1987; Wang *et al.* 1985; Wetterauer and Killorn 1996)

1.4.c. VAM Fungi in Australian Agricultural Systems

There are few field studies of VAM fungi in Australian agricultural systems. The most comprehensive work has been done by Thompson (1987; 1989; 1990; 1994a). Thompson (1987) provided an indication of the contribution of VAM fungi to crop growth in Queensland by comparing the growth of various paired crops, one crop growing poorly after long fallow, 'long fallow disorder', and the other growing better after a shorter fallow (Table 1.2). Crops growing after a long period of fallow had lower levels of VAM colonisation. Results from glasshouse trials where the VAM fungi were shown to improve plant uptake of P and Zn (Thompson 1987; Thompson 1994a; Thompson 1994b) supported the conclusion that, following a long fallow, the lack of VAM colonisation was responsible for reduced growth of the crops .

Table 1.2. Percentage of root length colonised by VAM fungi and shoot dry weight (g) in paired crops. One crop was preceded by a relatively short fallow — which had reduced VAM colonisation due to death of VAM propagules — and the other crop was preceded by a long fallow (adapted from Thompson 1987; various crop ages).

	VAM (%)		Shoot dry weight	
Fallow Period	Long	Short	Long	Short
Chickpea (*Cicer arietinum* L.)	18	73	0.5	2.9
Sunflower (*Helianthus annuus* L.)	3	14	0.6	6.9
Sorghum (*Sorghum bicolor* (L.) Moench)	17	70	6.6	51.4
Wheat (*Triticum aestivum* L.)	21	40	35.7	37.2
Maize (*Zea mays* L.)	16	57	0.8	6.5

The results of Thompson (1987, 1994a and 1994b) strongly indicate that the presence of VAM fungi enhances the growth of some crops, probably through increasing nutrient uptake. These results were obtained on cracking clay soils in the wheatbelt in NE Queensland and it is not known whether they would be applicable to the red and red-brown earths which predominate in the southern wheatbelt, where long fallow disorder has not been reported and where the farms sampled in this project were located. There are no Australian studies about the importance of VAM fungi for crop growth or production in more complex systems, such as pastures.

Other factors besides long fallow have been shown to significantly influence VAM colonisation levels in Australian agricultural systems. In particular, addition of soluble P fertilisers reduces VAM colonisation (Armstrong *et al.* 1992; Dann *et al.* 1996; Ryan *et al.* 1994; Thomson *et al.* 1992). While the influence of fungicides and herbicides on VAM fungi was listed as 'variable' in Table 1.1, the concentrations at which such chemicals are applied on the farms sampled in this project makes it unlikely that they would significantly influence VAM fungi (Ryan *et al.* 1994).

The contribution of VAM fungi to agricultural systems may not be reflected in short term crop yields. By influencing nutrient cycling (Jeffries and Barea 1994), contributing towards the maintenance of soil structure (Tisdall 1991), providing energy (hyphae) to the soil ecosystem and through interacting with pathogens (Thompson and Wildermuth 1989), VAM fungi may contribute towards the long term sustainability of agricultural systems (Bethlenfalvay and Schüepp 1994). Their presence may be vital if functional low-input agricultural systems are to be developed.

1.5. Difficulties with Assessing the Functioning of VAM Fungi in the Field

The characteristics of the VAM-host plant relationship are now generally accepted, particularly in regard to P uptake. However, field studies — especially in natural ecosystems — have seldom found the large growth benefits to the host plant from colonisation by VAM fungi that have been commonly found in glasshouse trials (Fitter 1986; Jensen 1983; McGonigle and Fitter 1988; Newbould and Rangeley 1984; Sanders and Fitter 1992). This may be due to both the manner in which VAM fungi actually function under field conditions, which implies that glasshouse trials are often not very relevant to field conditions, and/or difficulties associated with assessing the functioning of the symbiosis under field conditions. Some of these factors are discussed below, while further discussion is presented in section 12.1.b.ii.

Simply growing plants in pots will influence a range of factors — from the micro-organism communities present, to soil water and temperature regimes — which will affect growth of both VAM fungi and host plants. In addition, glasshouse trials are often conducted using sterilised soil and single isolates of VAM fungi. This simplifies interpretation of immediate results, but results are difficult to apply to more complex field conditions. For instance, in the field up to 12 species of VAM fungi may be present in an individual root system (J. Morton, pers. comm.). Sterilised soil lacks the micro-organisms and soil animals which may significantly influence interactions between plants and VAM fungi in the field (Daniels Hetrick *et al.* 1988; Gange and Brown 1992; Warnock *et al.* 1982). Soil sterilisation may also alter the pH and the availability of nutrients in the soil (Thompson 1990). Glasshouse trials often involve plant densities or species mixtures — both of which may influence functioning of VAM fungi (Allsopp and Stock 1992; Fitter 1977; Grime *et al.* 1987) — very different from field conditions. Moreover, glasshouse trials do not duplicate the effects of a hyphal network, which may be present in the field.

The variation in environmental parameters in the field, spatially and temporally, also makes it difficult to use results from glasshouse trials to predict responses in the field. For instance, light, temperature, soil water, soil nutrients and other soil micro-organisms may all influence the functioning of VAM fungi (Bolan *et al.* 1984; Manske 1989; Smith and Bowen 1979; Tester *et al.* 1986; Watkinson *et al.* 1996) and may vary greatly between sites in the field. Growth of plants in the field may be limited by pathogens or environmental factors other than P (Newsham *et al.* 1995) and may be on a seasonal cycle not present in a glasshouse. Thus the effect of VAM colonisation on plant growth in the field may be subtle and difficult to detect and may be important only at certain times of year (Dunne and Fitter 1989).

Perhaps the major problem in field research involves the difficulties inherent in producing a non-VAM control (Jakobsen 1994). Methods currently used — including

fungicides and fumigants — involve disturbance to the natural functioning of the host plant, as levels of available soil nutrients may be altered and the activities of other organisms, including pathogens (Thompson and Wildermuth 1989; Watkinson *et al.* 1996), will also be influenced. It may also be difficult to make a meaningful measure of the level of VAM functioning under field conditions. Unlike newly-colonised plants in glasshouse trials (see Smith and Gianinazzi-Pearson 1990) the percentage of root length colonised in the field may not reflect current mycorrhizal activity, as the proportion of the colonisation consisting of arbuscules may vary significantly over time (Dodd and Jeffries 1986) and not all arbuscules may be active. In addition, factors such as insect grazing may have affected the growth and nutrient absorbing ability of the external hyphae (Warnock *et al.* 1982).

Results from studies of agricultural systems do indicate that VAM fungi are important for nutrition in many crop species (§1.4). Conclusive results are easier to obtain from agricultural systems than natural systems, as many of the complex factors which make it difficult to assess VAM functioning in natural systems are absent or simplified. For instance, crops are typically fast growing, genetically-uniform, even-aged monocultures of even density and — providing there are no significant diseases present — the factor limiting yield will generally be soil nutrients, especially P and N (§2.2.a). Overall, the difficulties in collecting meaningful results from the field have resulted in relatively few studies and there is a general lack of knowledge about the occurrence and functioning of VAM in the field (Klironomos and Kendrick 1993). Yet, ultimately, the aim of most studies is to understand how VAM fungi function in ecosystems, not just in the glasshouse or laboratory.

1.6. Broad Ecological Trends

Due to their abundance in most ecosystems and their broad range of possible activities, VAM fungi may play a pivotal role in ecosystem dynamics through their influence on processes such as nutrient cycling and plant competitive interactions (Allen 1991; Watkinson *et al.* 1996). However, research is only just beginning to address the role of VAM fungi in ecological interactions in the field (see Allen 1991; Merryweather and Fitter 1995b; Watkinson *et al.* 1996). In particular, few studies have examined whether there exist broad ecological relationships involving VAM fungi.

A number of broad surveys of VAM occurrence have found the frequency of VAM fungi generally correlating negatively with the abundance of P or other soil minerals (Allsopp and Stock 1994; Mårtensson and Carlgren 1994; McNaughton and Oesterheld 1990), however these correlations were not experimentally linked with the activities of VAM fungi in the ecosystems.

If the roles of VAM fungi in broad scale processes — such as climate change (O'Neill *et al.* 1991), biogeochemical cycling (Miller and Jastrow 1994) or the effects of air pollution on ecosystems (Shafer and Schoeneberger 1994) — are to be calculated, it is necessary to identify any parameters that correlate to the broad scale occurrence of VAM fungi and, if possible, relate these to the role of VAM fungi in ecosystem functioning. This must involve investigation of VAM fungi at a range of functional, temporal and spatial scales (Allen *et al.* 1992; Miller and Jastrow 1994; O'Neill *et al.* 1991).

1.7. Project Aims

While much is known about the functioning of the VAM symbiosis under controlled conditions (§1.3), relatively little research has been conducted into the influence of VAM fungi on ecosystem functioning. In particular, there has been little attempt to link what is known about the functioning of VAM fungi at the scale of the individual plant with broader ecological processes. In part, this deficiency results from the difficulties inherent in conducting research into VAM fungi under field conditions (§1.5).

Two major challenges to understanding broad-scale ecological processes are the inherent complexity of natural systems and the difficulties associated with creating large, replicated, long-term field experiments in which the effects of manipulating key variables can be examined. Agricultural systems can meet many of the later requirements and present a range of ecosystems of differing complexity. Thus, this project investigated ecological trends involving VAM fungi, P and plant growth using paired farms under contrasting farm management strategies, conventional and alternative, which were utilised as replicated treatments differing in P inputs. The interactions between VAM fungi and P were a major focus of this project, as P is a major limiting nutrient in Australian agricultural systems (§2.2.a). The paired farms were examined in two contrasting agricultural commodity production systems in SE Australia; a dryland (mixed) cereal-livestock system and an irrigated dairy system. The mixed farms alternated between annual crop monocultures and annual pastures, while the dairy farms consisted of permanent, perennial mixed-species pastures. Thus, the two systems represent strongly contrasting ecological communities.

The major questions addressed by this project can be classified into three broad interrelated topics of investigation (Fig. 1.3). The first topic was the ecology of VAM fungi at the scale of interactions between the environment, VAM fungi and host plants. The factors controlling the level of colonisation by VAM fungi and the influence of VAM fungi on plant growth in crops and pastures were investigated, allowing conclusions to be drawn about the form of the relationship between host plants and VAM fungi. Both field-sampling and glasshouse trials were used to assess the effect of

Topic One: The VAM fungi-host plant relationship

1) Which environmental and farm management factors control the level of VAM colonisation?

2) What influence does colonisation by VAM fungi have on plant growth?

3) A mutualistic or parasitic relationship?

Topic Two: Broad ecological trends

1) Do VAM fungi function differently in cropping and pasture systems?

1) Are there predictable relationships between environmental constraints, concentration of nutrients in soil and plants, and levels of VAM colonisation occurring across farm management strategies and commodity production systems?

Area Three: Comparisons of VAM fungi between conventional and alternative farms

1) Do plants on alternative farms have higher levels of VAM colonisation?

2) Are plants on alternative farms more reliant on VAM fungi for nutrient uptake?

3) Do the soil processes associated with plant nutrient uptake on alternative farms differ substantially from those on conventional farms?

4) Is there a role for VAM fungi in increasing the sustainability of agricultural systems?

Figure 1.3. The three broad topics addressed by this project and the major questions investigated in each.

VAM colonisation on plant growth. The glasshouse trials were designed to remain as relevant to field conditions as possible. Moreover, they often assessed the impact of variations in factors — such as plant density — which may affect the relevance of glasshouse trials to field conditions.

The results from Topic One were used to address broader questions concerning the functioning of VAM fungi in pasture and cropping systems and the existence of broad-scale ecological relationships between environmental constraints, soil and plant nutrient concentrations, and the occurrence and activities of VAM fungi (Topic Two).

The use of paired conventional/alternative farms as replicated treatments allowed investigation of three claims commonly made by proponents of alternative agriculture (Topic Three). Namely, that in comparison to conventional farms alternative farms have a greater abundance of soil organisms; are more reliant on soil biological activity for plant nutrient acquisition; and will develop different ecological processes which may not be quantifiable using conventional scientific methodology (§2.3.c). Conclusions are also drawn about the role of VAM fungi in the development of sustainable agricultural systems.

1.8. Thesis Structure

The thesis is divided into five parts (A - E), each comprising one or more chapters. All chapters presenting results from field-work commence with a brief overview of the material contained in the chapter. The aims of the research presented in the chapter are then presented, followed by materials and methods specific to that chapter, results and discussion. Each chapter concludes with a brief summary of the major findings from the chapter.

Chapter Two in Part A provides a more detailed background to the agricultural systems and farm management strategies included in this project. In Part B, Chapter 3 presents details of materials and methods which were repeatedly used. Part C presents results from the mixed farms. Chapter 4 introduces the mixed farms and results from field-sampling of crops and pastures are presented in Chapters 5 and 6 respectively. Glasshouse trials further exploring the contribution of VAM fungi to wheat growth are presented in Chapter 7 and Chapter 8 contains results from sampling during the severe drought of 1994. Part D presents results from the irrigated dairy pastures. Chapter 9 introduces the dairy farms and results from field-samplings are presented in Chapter 10. Results from glasshouse trials using dairy farm soil are presented in Chapter 11. Part E consists of Chapter 12, a general discussion of all results in relation to the questions posed in Figure 1.3.

Chapter Two

Project Design

This chapter commences with an overview of the design of the project. Background information is given on the key factors which were incorporated into the design as broad-scale variables: soil nutrients and water; farm management strategy; and commodity production system.

2.1. Overall Project Design

This project involved sampling a number of adjacent paired farms under contrasting farm management strategies, conventional and alternative; the alternative farms being either organic or biodynamic (§2.3). The conventional farms received high P fertiliser inputs, while the alternative farms received low or no P fertiliser inputs. The farm pairs were examined in two contrasting agricultural commodity production systems; a dryland (mixed) cereal-livestock system and an irrigated dairy system (§2.4). The mixed farms were alternating between a number of years of annual crop monocultures and a number of years of annual pasture, while the dairy farms were dominated by irrigated permanent perennial mixed-species pastures. Farms of the same commodity production system and farm management strategy were regarded as replicates. Where appropriate, further experiments were carried out under controlled glasshouse conditions using field soils.

All the selected farms had been under their current management strategy for many years and so these farm agroecosystems were approaching, or had reached, a state of dynamic equilibrium between the applied management, resource harvesting and natural biological processes. This design, shown in Figure 2.1, allowed the long term effects of variations in farm management practices, particularly P inputs, to be examined on replicated sites. It also allowed comparisons between the functioning of conventional and alternative agricultural systems. Due to the different geographic distributions of the mixed and dairy farms, contrasts between these systems are made between the results summarised within the systems. This design is reflected in the statistical analyses used in the thesis and in the organisation of subsequent chapters.

2.1.a. The Use of Agricultural Systems as 'Treatments' in Ecological Experiments

There are a number of advantages to using agricultural systems, as opposed to natural systems, when attempting to quantify ecological processes. These include the relatively simple structure of agricultural systems, which makes them inherently more manipulable and useful for developing models of key processes, as well as the availability of technology and sites for imposing or measuring the effects of manipulations (Groffman 1996).

Much research on agricultural systems has involved internally replicated factorial experiments conducted at research stations or on commercial farms. Such experiments have provided valuable information on the response of these systems to variation in particular inputs or management practices. Agricultural field research may also involve establishment of experimental plots designed to mimic entire farm management strategies; a popular method for comparisons of conventional and

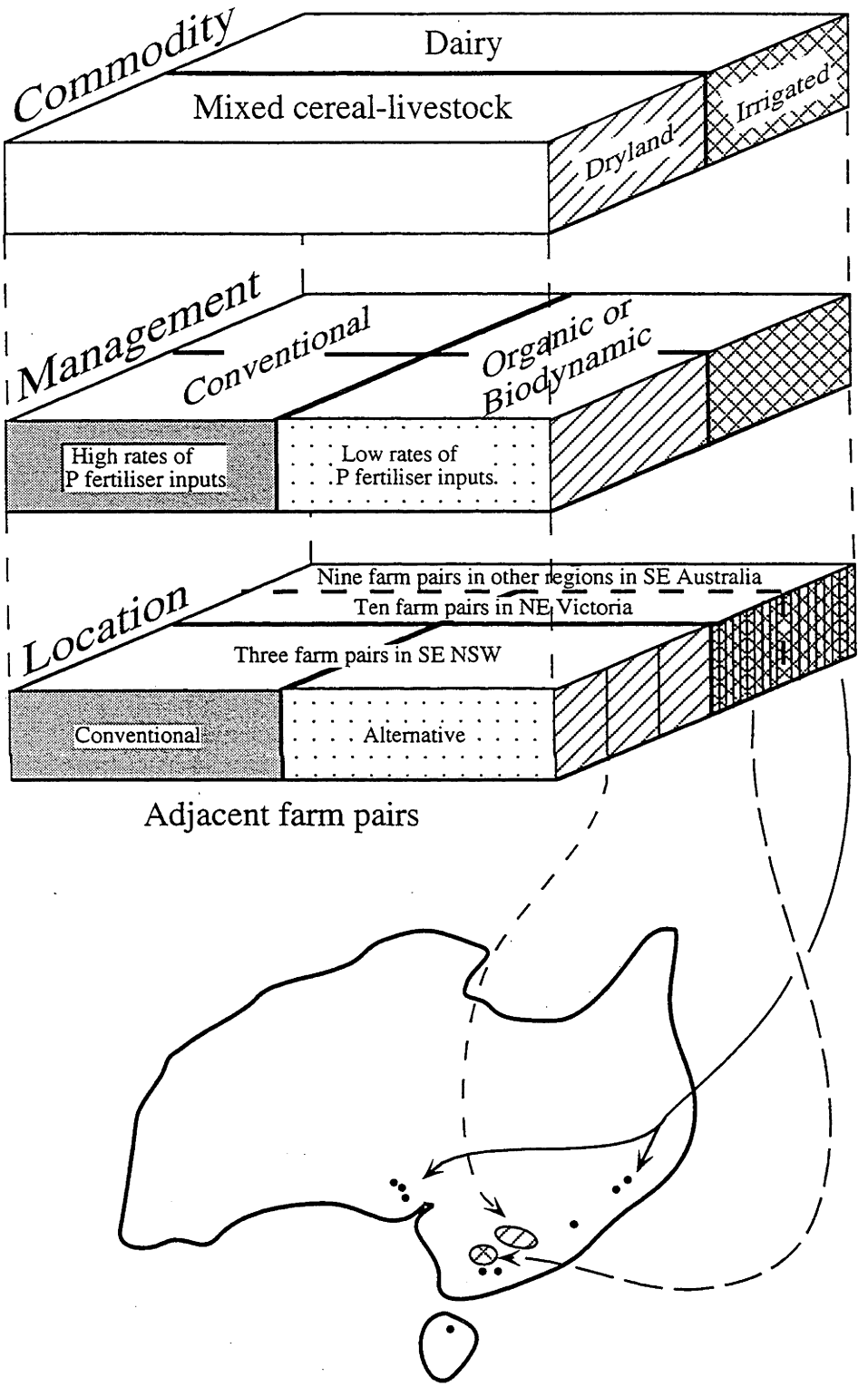


Figure 2.1. A broad illustration of the overall design of the project, comprising adjacent farm pairs of contrasting farm management strategy within geographic regions of SE Australia in which different commodity production systems predominate.

alternative systems (Penfold *et al.* 1995; Poulton 1995). Surveys of commercial farms have also provided useful information.

This project contains elements of all these approaches. It can be considered as a large-scale field experiment where a set of treatments — conventional or alternative management strategy and mixed or dairy system — were applied by farmers over a period of up to 40 years. Selected elements from the resulting farm ecosystems were then surveyed on 46 farms over a variety of time spans ranging from a single sampling, sampling over a (six month) growing season, or repeated sampling for up to four years. The four particular strengths of this approach are listed below.

1) Sampling from sites on long-established farms — as opposed to establishing short-term trial sites — makes it more likely that sites are at a dynamic equilibrium truly representative of the farming systems, rather than a transitional situation in which past influences and newly initiated changes may be confounded together (van Bruggen 1995). For instance, the functioning of alternative farms after conversion from conventional management has been reported to pass through a transition stage of 5-6 years during which time yields decrease as soil processes and plant growth reach a new equilibrium (Hassall and Associates 1996; Janke *et al.* 1991).

The repeated sampling of a number of sites over four years provided the opportunity to evaluate whether the farms sampled in this project had indeed reached an equilibrium, as well as allowing the effects of environmental variation, particularly drought, to be observed.

2) The use of adjacent matched farm pairs is advantageous as the pairs can be chosen to have similar underlying environmental conditions such as soil type, aspect, temperature and rainfall. Thus, differences detected between the two farms can be attributed to the known management differences. This approach has often been used in research into alternative agriculture (Elmholt and Kjøller 1989; Lockeretz *et al.* 1980; Reganold 1988 and 1995; Reganold *et al.* 1993; Small and McDonald 1993; Werner *et al.* 1990; Wynen and Edwards 1988). In this project, the farm pairs were generally treated as blocks for the purposes of statistical analysis.

3) By sampling 46 farms located over an area of southern Australia ranging from northern NSW to South Australia (Fig. 2.1), this project encompassed both slight variation in management practices under each management strategy as well as variation in environmental factors. This increases the robustness of any relationships observed in terms of generality, realism and independence (Shennan *et al.* 1991). Indeed, this project examined around 30% of the alternative mixed farms and > 90% of alternative dairy farms in SE Australia.

4) Using farms as treatment replicates, with sampling at the paddock level, allows a realistic examination of community level interactions. Interactions generated by the entire system, such as border effects of surrounding paddocks on the farm or ecological effects of whole farm changes, will be accounted for; these may not be present in trial plots (van Bruggen 1995).

The main weaknesses of this design are the problems inherent in matching and replicating sites in the field and the difficulties in confidently defining relationships between variables when a number of factors differ between treatments. The effects of these problems were minimised in this project through the pairing of conventional and alternative farms and by sampling a large number of farms. Although, as noted above, comparisons between farming systems in different regions are inherently less robust due to possible confounding processes. In addition, field data were used to formulate hypotheses which, when appropriate, were tested in glasshouse trials designed to isolate particular effects or interactions, whilst remaining as relevant to field conditions as possible.

2.1.b. The Role of Glasshouse Trials

Glasshouse trials are superior to field trials in a number of respects. In particular, they allow manipulation and regulation of independent variables and can be easily replicated. For instance, much of the current knowledge about VAM fungi has been generated from glasshouse trials involving sterilised potting mixes with single isolates of fungi (§1.3). The main weaknesses of glasshouse trials are their limited temporal and spatial scale and inability to recreate the conditions and the many interactions that occur in the field (§1.5). Thus they may lack realism and broad applicability, and their relevance to field conditions may be limited.

The influence of VAM fungi on plant growth is particularly difficult to study under field conditions due, in part, to the problems associated with creating non-VAM controls (§1.5). These difficulties, along with a lack of resources for sterilising soil in the field and the fact that sterilisation techniques would not be permitted under alternative farming standards (§2.3), meant that the influence of VAM fungi on plant growth was assessed in this project using glasshouse trials.

2.2. Environmental Factors Shaping Australian Agricultural Systems

Many factors, including numerous forms of land degradation, constrain agricultural production in Australia. Two factors which are deliberately manipulated by many farmers are soil nutrient concentrations, particularly P and N, and soil water. In SE Australia these are expected to exert a strong influence on both plant growth and the functioning of the relationship between plants and VAM fungi. Nutrients and water were incorporated into the project design as shown in Figure 2.1. Nutrients through inclusion of the two farm management strategies, and water through the inclusion of the two commodity production systems (although the effects of water are confounded with commodity system), as well as by the sampling of dryland mixed farms over several years of varying rainfall, including during the 1994 drought.

2.2.a. Soil Nutrient Concentrations

Australian soils have been shaped by a number of important influences: the long period of relative geological stability of the Australian continent; the small extent of Quaternary glacial activity; the generally low elevation and flat terrain; and the current dry climate. Many Australian soils are therefore very old. That is, they have developed on surfaces which have been subjected to weathering for millions of years (Lindsay 1985). As a result, the proportion of certain soil types is unusual and differs from the Northern Hemisphere; in particular, the proportion of nutrient-impoverished soils is exceptionally high (Lindsay 1985). Consequently, growth of plants in agricultural systems is often limited by nutrients, particularly P and N.

(i) Phosphorus

Phosphorus is an element required in relatively large amounts for plant growth. It is necessary for a variety of cell functions including cell division and growth, photosynthesis, sugar and starch formation, and energy transfer. Phosphorus is extremely chemically reactive and always exists as phosphate minerals in nature. These vary greatly in their solubility, but tend to transform from sparingly soluble to increasingly insoluble forms over time (Holford 1997). Consequently, P is the most immobile, inaccessible and unavailable of all nutrient elements in soil (Holford 1997). Soil P occurs in many different forms, which vary between different soil types, and no single component of it can be identified as 'plant-available' P (Holford 1997).

In Australian soils, both total and available P concentrations are generally low, except where soils have developed on more recent basalt or basalt derived alluvium. The extractable P concentration is usually 10-400 $\mu g\ g^{-1}$, and sometimes as low as 1 $\mu g\ g^{-1}$; in contrast, the majority of American and English soils have extractable P > 500 $\mu g\ g^{-1}$ (Lindsay 1985). The effects of low soil P are exacerbated by its frequent

immobilisation. Soils which contain high concentrations of iron and aluminium oxides are widespread in Australia and P combines readily with these oxides, becoming unavailable to plants (Lindsay 1985). As a consequence, in comparison to North America and Europe, Australia uses an unusually high proportion of P fertilisers relative to N or potassium (K). However, due to the less intensive nature of agriculture in Australia (§2.3.a), the rate of fertiliser application is generally much lower (McLennan 1996).

A generalised P cycle for an agricultural system is presented in Figure 2.2. Inputs of P are largely from applications of P fertilisers and outputs are mainly due to the removal of animal or plant biomass, although P may also be removed through the erosion of top soil (McLennan 1996). Leaching of P from soil generally does not occur in substantial quantities, as soluble P fertiliser is mostly immobilised close to the soil surface as highly insoluble precipitates. Indeed on many Australian soils, plant uptake of soluble P from fertilisers in the year of application is only around 20% (Leeper and Uren 1993). Consequently, in many areas, total soil P concentrations are increasing, as more P is added in fertilisers than is removed in produce (Leeper and Uren 1993). However, yields in many areas of Australian agriculture are strongly dependent on regular additions of P fertiliser to supply P in a form that plants can access (Holford and Doyle 1992). Thus, it is likely that VAM fungi aid plants in accessing P from soils in Australia, however the importance of their contribution has not been well-quantified under field conditions (§1.5).

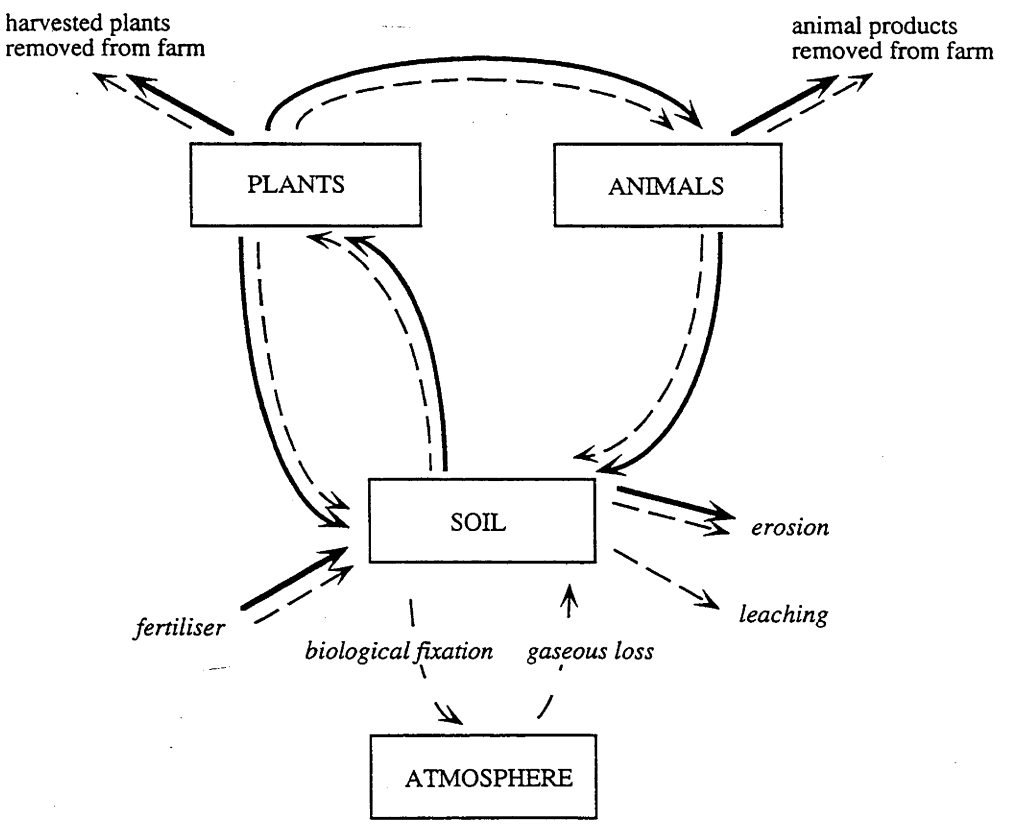


Figure 2.2. Generalised diagram of major pathways in the cycling of N (— —➤) and P (——➤) in an agricultural system.

Phosphorus is generally applied to crops and pasture as superphosphate (9-19% P), ammonium phosphates (20-22% P, 12-18% N), compound N-P-K fertilisers, or rock phosphate (11-16% P). Rock phosphate is the raw material from which the other, more soluble, fertilisers are manufactured through the addition of sulphuric acid producing the water-soluble acid phosphate $Ca(H_2PO4)_2$. Rock phosphates differ widely in their mineral constituents and have been rather arbitrarily divided into 'reactive' and 'unreactive', where reactive rock phosphate fertilisers have > 30% of P soluble in 2% citric acid (Bolan *et al.* 1990). Except on soils based on highly leached sands, both reactive and unreactive phosphate rocks have been found, in Australia, to have a low effectiveness relative to superphosphate, due to their low solubility (Bolan *et al.* 1990).

(ii) Nitrogen

Nitrogen is an essential constituent of proteins, including the enzymes in the photosynthetic process and is required in large amounts relative to the other essential elements. Consequently, the photosynthetic capacity of a crop or pasture is often very closely related to N supply.

While Australian soils also tend to contain low levels of N, N differs from P in potentially being available to a system through biological fixation from the atmosphere (Campbell and Bowyer 1990). A generalised N cycle for an agricultural system is presented in Figure 2.2. Nitrogen, in the form of N_2 gas, is the largest component of the atmosphere, but plants cannot directly access N_2 as they may only absorb N in ionic forms such as NO_3^-, NO_2^- and NH_4^+. However, N_2 may become available to plants through the actions of N-fixing micro-organisms, particularly bacteria in the genera *Rhizobium* and *Bradyrhizobium.* These bacteria may be free-living in the soil or may form a symbiotic relationship with plants of the legume family. *Rhizobium* converts N_2 from the atmosphere into organic N compounds, almost all of which are available to the host plant. In addition to being removed in agricultural produce, unlike P, N is constantly lost from soil as NO_3^- in drainage water and as N_2 gas or N-oxide through denitrification (Leeper and Uren 1993).

There are many types of N fertilisers and their composition varies greatly. Two common types are urea (46% N) and mixed N-P fertilisers such as diammonium phosphate. These fertilisers use synthetic ammonium, produced as a by-product from industrial processes such as coal-coking, as their major N source (Reid 1990).

2.2.b. Water

Australia has a generally dry climate characterised by hot summers and mild winters. The average annual rainfall in Australia of 410 mm is lower than that of any other continent (Davidson 1969). In addition, due to the high levels of radiation received in

Australia, rates of evaporation and transpiration tend to be high. In combination with soils of low porosity which do not store water well, this results in effective rainfall for crop and pasture production often being lower than on other continents (Ockwell 1990).

A further important feature of the Australian climate is the variability in rainfall and the occurrence of drought. There were nine major droughts and about 30 severe regional droughts in the 100 years from 1890 to 1990 (Ockwell 1990). This variability relates to interaction between, and movement of, the monsoon subtropical high and high latitude-belt westerly systems.

(i) Dryland Farming

Around 80% of the total value of agricultural produce in Australia is produced by dryland (non-irrigated) agriculture (McLennan 1996). This includes most of the production from mixed cereal-livestock farms in the southern and northern wheatbelt. The lower limit of rainfall for wheat production, assuming optimum temperatures and radiation, is about 225 mm during the wheat growing season (Hamblin and Kyneur 1993). The mixed farms sampled in this study all had an average growing season rainfall well above this minimum. When rainfall is above average, wheat becomes significantly limited by other factors, with yields up to 50% below their potential (Cornish and Murray 1989). These other factors include nutrients and disease.

During the 1994 drought, growing season rainfall was 61-147 mm on the farms studied in this project, providing an opportunity to examine the functioning of these farms under conditions of severe water stress. Even during years of above average rainfall, dryland systems differ from irrigated systems which have more regular inputs of water and therefore experience less variation in soil water content.

(ii) Irrigated Agriculture

Currently in Australia, 70% of total water use is by irrigated agriculture, which produces around 20% of the total value of agricultural product (McLennan 1996). Of the 2.4 million hectares of irrigated land in Australia, 1.4 million are under pasture and are grazed to produce fat lambs, beef and dairy produce (McLennan 1996). This contrasts with other countries where irrigated land is normally used for crop production (Davidson 1969).

Irrigation allows establishment of perennial pastures, such as the ones sampled during this project on the dairy farms in NE Victoria, as production can be maintained throughout the year. However, environmental problems associated with irrigation — including rising water tables and salinisation on farms — along with deteriorating downstream water quality due to factors such as eutrophication, are rapidly increasing. These may seriously reduce the lifespan of irrigation schemes (Mussared 1995).

2.3. Farm Management Strategies

This section contains a brief description of conventional agriculture in Australia, concentrating on characteristics unique to Australia, before providing more detailed background information on organic and biodynamic agriculture, as these alternative forms of agriculture may be unfamiliar to many readers.

2.3.a. Conventional Agriculture

As in other developed countries, Australian agriculture is highly industrialised with high levels of mechanisation and large quantities of fertilisers, pesticides, and herbicides being used commonly over many decades. However, it differs from industrialised agriculture in regions such as Europe and North America in some aspects.

Australia's agricultural systems have been primarily shaped by climate and population size. Only 25% of Australia has sufficient rainfall for a growing season > 5 months and only 9% has a growing season > 9 months. Consequently, only a small proportion of the continent has a climate capable of sustaining crops and improved pastures (Davidson 1990). However, Australia has only a small population resulting in land being cheaper, and labour more expensive, than in other developed countries and providing only a limited domestic market. Consequently, profitable agricultural industries in Australia tend to have the following characteristics: utilisation of a large area of land; minimum use of labour; production of commodities which have export markets; and production of commodities capable of being transported large distances without deteriorating (Davidson 1990). Australia's largest agricultural industries are broad-scale production of wheat, wool, sheep meat and beef.

Individual Australian farms generally produce several products which are characterised by complementary and substitution type relationships in resource use and enterprise combination (Ockwell 1990). The mixed farms studied in this project are a typical example. Further detail on conventional agriculture in Australia can be found in many publications including Reid (1990) and Campbell and Bowyer (1990). A comparison between the management practices of conventionally and alternatively managed farms is presented in Table 2.1 and further details on the management of the farms sampled in this project are presented in Chapters 4 and 9.

2.3.b. Alternative Agriculture

In Australia the two dominant and well-defined alternative systems of agriculture are organic and biodynamic farming. Although they currently account for < 0.8% of agricultural land area, this is expected to increase to 1.9% by 2005 (Hassall and Associates 1996). Both organic and biodynamic farmers follow codes of practice

Table 2.1. Generalised comparison of the three main farm management strategies used in Australian agriculture.

	Conventional	Organic	Biodynamic
Background	Based on European agriculture. Modified by modern Australian agricultural research.	Arose out of conventional agriculture. Often a reliance on techniques developed by individual farmers.	Emerged in Europe during the 1920's. Based on teachings of the Austrian philosopher Rudolph Steiner.
Philosophy and Principles	Emphasis largely on maximising production and profits. Increasing awareness of environmental responsibilities.	Along with economic returns, emphasise improving environmental quality and maintaining the land for future generations. Aim to produce nutritional and healthy produce.	Within the constraints of economic viability aim for a diverse, site-adapted, self sustaining system, with minimum inputs and outputs. Emphasis on improving soil fertility through stimulating soil micro-organisms. Aim to produce nutritional and healthy produce.
Weed Control	Synthetic chemical herbicides. Tillage. Rotations.	Tillage. Rotations and crop planting times. May tolerate higher weed levels. Do not use synthetic herbicides.	Tillage. Rotations and crop planting times. May tolerate higher weed levels. Do not use synthetic herbicides.
Pest and Disease Control	Synthetic chemical pesticides. Rotations. Resistant varieties.	Rotations. Resistant varieties, breed resistance through selection on-farm and enhance resistance through balanced nutrition. Biological control /encouraging natural predators. Do not use synthetic chemicals.	Rotations. Resistant varieties, breed resistance through selection on farm and enhance resistance through balanced nutrition. Biological control/encouraging natural predators. Use of homeopathic preparations. Do not use synthetic chemicals.
Plant Nutrition	Soluble chemical fertilisers supply P, N and other elements. Nitrogen from legumes.	Relatively insoluble mineral bearing rocks supply P and other elements. Nitrogen from legumes. Composted animal manures. Do not use synthetic soluble chemical fertilisers.	If apply fertilisers use relatively insoluble fertilisers at low rates to supply P and other elements. Nitrogen from legumes. Composted animal manures. Homeopathic preparation BD500 is used to stimulate soil micro-organisms Do not use synthetic soluble chemical fertilisers.

which are produced by various certifying organisations and which comply with national standards produced by the Australian Quarantine and Inspection Service (AQIS). According to AQIS (1997), the principle aims of alternative agricultural systems in Australia include:

- the production of food of high nutritional value;
- the enhancement of biological cycles in farming systems;
- maintaining and increasing fertility of soils;
- working, as far as practicable, within a closed system;
- the avoidance of pollution resulting from agriculture;
- minimising the use of non-renewable resources; and,
- the co-existence with, and the protection of, the environment.

The management of alternative farms in Australia may vary markedly depending on the underlying goals of the farmer and the constraints imposed by economic, social and environmental factors. However, these farms do share certain management features which are the consequence of farmers striving for the aims listed above, within the rules defined by the certifying organisations. Many of these features are listed in Table 2.1 and the most definitive, which are not necessarily exclusive to alternative agriculture, are listed below:

- avoidance of manufactured chemical herbicides and pesticides;
- reliance on tillage for weed control;
- avoidance of highly soluble fertilisers such as superphosphate;
- if P fertiliser is used, it is generally the relatively insoluble rock phosphate;
- inclusion of legumes in the rotation to provide N;
- use of rotation, culling and mineral licks to control animal parasites;
- retention of crop stubble rather than burning;
- on-farm processing of produce to add value and/or sell directly to processors or consumers.

As both organic and biodynamic farms were sampled in this study, the principles behind each management strategy and their basic management practices are briefly described below.

(i) Organic Agriculture

Organic farmers in Australia use a diverse range of management strategies which enable them to operate without the use of soluble fertilisers, pesticides, herbicides and other biocides (Wynen 1992). Supplying nutrients in a soluble form is considered to result in the plants absorbing excessive quantities of nutrients with negative consequences for the health of the plants and the animals or humans consuming them.

Nutrients are therefore supplied in poorly soluble forms in the belief that the plant will absorb these nutrients only when they are necessary for 'healthy' growth (Madge 1995). Consequently, mineral rocks are used to supply nutrients and P is added as the relatively insoluble rock phosphate. Organic farmers do not apply N fertilisers, as N can be acquired from the atmosphere through the actions of *Rhizobium* bacteria. Rotations on mixed farms may be adjusted to maximise the contributions of legumes by, for example, inclusion of longer pasture phases between crops (Wynen 1992).

Pesticides, herbicides and other biocides are replaced with a range of management strategies listed in Table 2.1. Crop stubble is generally retained, as opposed to being burnt, as it is considered to supply energy for the functioning of soil micro-organisms. Currently on the mixed and dairy farms examined in this project, there are generally no pests with the potential to inflict major economic damage, although weed levels in crops may be high enough to significantly reduce yield and profits.

Additional information on organic agriculture in Australia can be found in Derrick (1996), Dumaresq *et al.* (1997), Madge (1995) and Wynen (1988 and 1992).

(ii) Biodynamic Agriculture

Despite some differences in philosophy, the management practices of Australian biodynamic farmers are essentially similar to those of organic farmers, that is, no use of manufactured biocides or soluble fertilisers, while employing alternative methods of pest and weed control (Table 2.1). However, while organic farmers may apply relatively insoluble mineral fertilisers at similar rates to conventional farmers, biodynamic farmers rarely apply fertilisers and when they do, it is at extremely low rates. Biodynamic farmers also place greater emphasis on diversification and this may be reflected in a diverse range of crops and livestock being farmed and a tolerance of weeds.

The philosophy and practices of all biodynamic farmers originate from a single source; the teachings of the Austrian philosopher Rudolf Steiner in the early 1920s. Steiner claimed that modern science can not fully explain nature, as it overlooks the spiritual aspect of reality (Schilthuis 1994). Thus biodynamic farmers hold the belief that "*every being has a link with the spiritual cosmic world*" and consequently the role of the farmer is to ensure that "*these links can take place undisturbed*" (Schilthuis 1994). Both the farm and the earth are viewed as one living organism. Harmony with nature is sought and farmers aim for their farms to be closed and self-sufficient, although achieving this aim is obviously limited by the need for commercial viability.

These beliefs result in a number of management practices unique to biodynamic agriculture, including planting by the phase of the moon and the use of various biodynamic (homoeopathic) preparations which, it is claimed, stimulate and regulate

complex life processes (Kolisko and Kolisko 1978; Schilthuis 1994). The preparation most widely used by Australian biodynamic farmers is BD500, which is made from cow manure composted over winter in a buried cow horn and applied, after appropriate stirring, with the aim of stimulating soil life. BD500 contains approximately 0.7% P and 2% N (Nguyen and Haynes 1995), along with various micro-organisms and is applied at the rate of 1-2 g ha^{-1}. For the biodynamic farmer, and most organic farmers, soil fertility is considered to be more than the presence of nutrients in certain quantities. It is believed that nutrient deficiencies in plants are due not to a lack of nutrients in the soil, but a lack of availability. Consequently, emphasis is placed on enhancing microbial activity as a means to improve soil fertility by developing humus and increasing the availability of nutrients (Small *et al.* 1994a). In contradiction to conventional science, it is also believed by some biodynamic farmers that transmutation of elements is possible (Podolinsky 1989). Put simply, on a properly-functioning biodynamic farm, P in an 'alive-form' — such as P in the microbial biomass — is thought to be capable of creating P from more common elements when P is needed for plant growth.

Information on biodynamic agriculture in Australia can be found in Podolinsky (1985 and 1989), Small *et al.* (1994a, b) and Wynen (1994a), while more general information is contained in Kolisko and Kolisko (1978), Schilthuis (1994), Steiner (1993) and Reganold (1995).

2.3.c. Soil Biological Activity on Conventional and Alternative Farms

The use of paired conventional and alternative farms as treatment replicates allowed examination of some of the claims made by advocates of alternative agriculture. In particular, it is often claimed that increased soil organism activity is critical to the successful functioning of alternative farms (La Rooj 1989; Lopez-Real 1985; Macgregor 1994; National Research Council 1989; Penfold *et al.* 1995; Sinnamon 1996; Wynen 1992). Indeed the Australian National Standard for Organic and Biodynamic Produce states that such produce is defined by being "*produced in soils of enhanced biological activity, determined by the humus level, crumb structure and feeder root development, such that plants are fed through the soil ecosystem and not primarily through soluble fertilisers added to the soil*" (AQIS 1997).

This statement implies that a particularly important role of soil organisms on alternative farms is to supply plants with nutrients from relatively insoluble sources in the soil. Moreover, it implies that soil biological activity might be either lower, or of little significance, on conventional farms. It has also been suggested that the biological processes present on alternative farms differ so greatly from those on conventional farms, that the current ability of science to describe these processes may be limited (Wynen 1996). Such claims are frequently made, in spite of little supporting evidence

(see Wander *et al.* 1995 and the review by Ryan 1997 presented in Appendix 6). This project provided an opportunity to test their applicability in two contrasting agricultural commodity production systems.

2.3.d. Conventional and Alternative Farms as Nutrient Addition Treatment Plots

The conventional mixed and dairy farms sampled during this project applied soluble P fertilisers at up to 30 kg ha^{-1} $year^{-1}$ of P. On some alternative mixed farms, P was applied at a similar rate as the less soluble rock phosphate or reactive rock phosphate. However most alternative farmers, particularly the biodynamic dairy farmers, applied very low rates of rock phosphate or applied no P fertiliser. Alternative farmers relied on biological fixation of N by legumes for supply of N, while conventional farmers generally applied fertilisers containing N; particularly diammonium phosphate, superphosphate, or urea.

The differences in P applications between the conventional and alternative farms were more consistent than those of N and were also easier to measure, as significant P inputs can only be in the form of fertiliser applications, while N may also enter a system through biological fixation. Hence, the conventional/alternative farm pairs were treated primarily as large-scale, long-term, field treatments differing in P additions.

2.4. Commodity Production Systems

This project involved sampling from two distinctive commodity production systems; dryland mixed farms and irrigated dairy farms. These were chosen over other commodity production systems present in SE Australia for a number of reasons:

- the permanent perennial pasture on the dairy farms differed markedly in a number of aspects from the annual crops and pastures on the mixed farms, allowing comparisons to be made between two contrasting biological systems;
- both systems had conventional and alternative farms available for study;
- both represent significant means of agricultural production in Australia;
- dairy farms located across four states in SE Australia were available for sampling, allowing comparisons to be made between farms on very different soil types and in quite different environments (see maps in Figs. 2.1 or 9.1).

2.4.a. Mixed Farms

Mixed farms are the predominant agricultural enterprise throughout much of inland SE and SW Australia. There are around 18 000 farms of this type in Australia, average size 1 400 ha, over a third of which are in New South Wales (ABARE 1995). Paddocks on these farms are rotated between crops and pastures, with approximately 20% of farm

area sown to crops each year (ABARE 1995). Crops grown are predominantly cereals, but also include grain legumes and oilseeds. Annual pastures of legumes and grasses support sheep for wool and meat, and beef cattle. This project sampled mixed farms from the SE NSW wheatbelt (Figs. 2.1 or 4.1). More details about these farms are presented in Chapter 4.

2.4.b. Dairy Farms

Australian dairy farms are relatively intensive operations in comparison to the mixed farms. The farms are based on permanent pastures of legumes, generally clovers, and grasses. There are around 14 000 dairy farms in Australia, averaging 180 ha in size. Of these farms, 84% are located in the eastern states with 56% in Victoria (ABARE 1995). Many of the Victorian dairy farms are located in the irrigation regions around the Murray and Goulburn Rivers. Slightly over 50% of dairy farms in Australia are irrigated (Watson *et al.* 1983). This project involved sampling farms across SE Australia. These farms maintained permanent pastures which had not been tilled for 8-40 years and usually had both perennial pastures — which were regularly irrigated during summer and which were the focus of this project — as well as non-irrigated annual pastures. More details about the dairy farms sampled in this project are presented in Chapter 9.

Ecologically, the mixed farms are quite different to the dairy farms, particularly in regard to the frequency and severity of perturbations and disturbance. On mixed farms, paddocks alternated between 1-3 years cropping with annual monocultures and 3-8 years annual pasture. The transition between pasture and crops was dramatic with most living plants being killed by tillage or herbicides in spring and the soil being left bare for 2-8 months over summer (a summer fallow). A number of cultivations, addition of fertilisers — and, on conventional farms, biocides — then occurred around the sowing of a crop in autumn. Thus, during the cropping phase, an annual cycle of summer fallow, tillage, sowing, crop growth and harvest was imposed. In contrast, the dairy pastures were managed with the aim of sustaining shoot production, and milk production, all year. This was achieved through eliminating tillage and managing perturbations such as grazing, addition of fertilisers and summer irrigation, as well as by maintaining a mixed-species pasture which actively grows all year. The dairy pastures had not been tilled, and therefore had persisted, for an average of 40 years. Thus, it is likely that the roles of VAM fungi differ between the mixed and dairy systems.

Part B

General Materials and Methods

Chapter Three

General Materials and Methods

To avoid repetition, this chapter details materials and methods which are relevant to more than one chapter. Methods specific to a particular chapter are provided in that chapter. An explanation of the protocols used for data presentation and statistical analysis is given at the end of the chapter, along with information about the extent of collaboration with other researchers.

3.1. Choosing Farms and Paddocks to be Sampled

Whenever possible, sampling occurred simultaneously on a conventional/alternative farm pair (§2.1). The conventional farm was chosen to be as similar to the alternative farm as possible in terms of environmental factors, such as soil type and aspect, as well as size and types of commodities produced.

The paddocks sampled on each pair were carefully matched, first in terms of their stage in the farm rotation and then, as closely as possible, in terms of environmental variables such as soil type and aspect, and pasture composition in the case of pasture paddocks. While a crop could always be repeatedly sampled during the growing season, it was not always possible to repeatedly sample the same paddock on the dairy farms due to factors such as irrigation and the type of stock being kept in the paddock. In such cases, a neighbouring paddock was chosen for sampling.

General locations of the farms sampled are given in each chapter, however in order to ensure confidentiality for the farmers who participated in this project, exact locations of individual farms and the names of farmers are not provided.

3.2. Field Sampling Methods

For assessment of the level of VAM colonisation in a crop, individual plants with their root systems attached were collected. In each paddock, plants were collected from 10-20 randomly selected sites which were > 25 m apart, > 10 m from the edge of the crop and excluded margins near fences, vehicle access tracks, fire breaks around crops and areas close to trees. A spade was used to remove a rectangle of soil, approximately 200 x 100 x 150 mm deep. This included up to 10 plants. Samples were placed in plastic bags and after returning to the laboratory, stored at 4°C until processing when each sample was placed in a bucket of water and soil gently teased from the roots. Of the plants then separated, one would be randomly selected, the roots removed and stored in 80% ethanol and the shoot dried at 70°C.

Pasture paddocks were sampled at 15-20 sites > 15 m apart, along a rough diagonal transect of the entire paddock. At each site a sample was taken as described above for the crops. To minimise disturbance to the relatively small dairy paddocks, it was ensured that all pasture species to be examined for VAM fungi — for example, clover and the predominant grass species — were present in the one sample. Samples were stored as described above for crops, before being washed in water and individual clover and grass plants extracted. The roots of each species at each site were stored separately in 80% ethanol. The sample for each site for a particular species contained root systems or shoots from a number of plants, as one plant often did not provide an adequate amount of material. If shoots were kept, they were dried at 70°C. If soil

samples or shoot material for nutrient analysis were collected at the same time as the roots, they were taken from the same sites in each paddock.

Owing to the difficulties with permanently marking sites in paddocks on commercial farms, when paddocks were sampled more than once as part of a time series, new sites were selected. The sampling procedures described above were considered appropriate for obtaining independent unbiased samples typical of each paddock.

3.3. Plant Shoot Measures

3.3.a. Crop Biomass, Development and Yield

Crop growth is usually presented as individual shoot weights and final yield. An individual plant shoot was collected from each site sampled in each paddock, dried at 70°C for at least 48 hours and weighed. Crop yield figures, based on volume of grain delivered to the silo, were obtained from farmers. Crop development stage was assessed using the Zadoks score (Zadoks *et al.* 1974).

Sampling of crops in 1993 (Chapter 5) was made in conjunction with Derrick, who made more precise measurements of crop biomass and yield (see Derrick 1996). At each site, all shoot material inside a 300 x 300 mm quadrat was removed, separated into crop and weeds, dried at 65°C for 24 hours and weighed. Crop yield was estimated by hand threshing the grain from plants in each quadrat and weighing each sample. No measures of plant growth were made on pastures.

3.3.b. Plant Nutrient Concentrations

Determination of P and N concentrations in shoot and root material was carried out in the Soils Analysis Laboratory in the Forestry Department at the Australian National University, following the method of Heffernan (1985a). All plant material had been dried at 70°C for 48 hours. When individual samples were heavier than 0.15 g they were ground, using a coffee grinder, to fragments < 1 mm to obtain a representative subsample. Samples that weighed < 0.15 g were cut using scissors into approximately 5 mm^2 sections. Approximately 0.15 g was taken from each sample and the exact weight noted. Five ml of digestion acid was added (300 g potassium sulphate 1 sulphuric $acid^{-1}$) and samples heated at 100°C for 20 minutes before 1 ml of potassium peroxide was added and the temperature raised to 380°C. After an hour, 1 ml of potassium peroxide was added and after another hour, 0.5 ml peroxide added. Samples were kept at 380°C until all were totally digested and, after cooling, they were analysed for N and P using automated spectrophotometry.

Unless noted in the methods section of an individual chapter, plant samples from each of the 10-15 sites sampled in a paddock were analysed separately to allow calculation of a mean and give an indication of variation.

Some measures of shoot nutrient concentrations in 1993 crops (Chapter 5) were provided by Derrick (see Derrick 1996). Crop shoot material from a 300 x 300 mm quadrat at each site was dried for 24 hours at 65°C and ground in a grinding mill. Analysis for P concentration was conducted using X-ray fluorescence spectrometry. Analysis for N concentration followed the method of Heffernan (1985a), described above. Some measures of pasture nutrients on the dairy farms (Chapter 10) were provided by Small (see Small *et al.* 1994b). Pasture was cut to ground level at 60 random sites on each farm, dried at 60°C and mineral concentrations measured (exact methods not specified).

3.4. Plant Root Measures

3.4.a. Root Length

For measurement of crop root length, a cylindrical core — 50 mm diameter and 50 mm deep — was taken from a randomly selected site along the crop row and a second core from the centre of the inter-row was taken immediately adjacent. The results from these were averaged to give a measure of root length at each sampling site (Kumar *et al.* 1993). When cores were taken at a number of depths, they were taken immediately below each other. Cores were stored at 4°C for no more than one week before being soaked in water for 24 hours and washed on a 2 mm sieve. Roots and organic matter remaining on the sieve were stored in 80% ethanol.

Root length was estimated using the gridline intersect method on a 12.73 mm grid under a dissecting microscope at x15 magnification (Giovannetti and Mosse 1980). Although samples were again washed on a 2 mm sieve before counting, a large amount of organic material remained; particularly subterranean clover (*Trifolium subterraneum* L.) burrs and decomposing crop shoot material. This was ignored during counting. Dead roots — distinguished by their shrivelled appearance and darker colouring — were also ignored, and thus a measure of active root length was obtained. It was not possible to consistently distinguish between the roots of the various species that were present in some paddocks, for example wheat (*Triticum aestivum* L.), wild oats (*Avena fatua* L.) and rye grass (*Lolium rigidum* Gaud.) were sometimes all common. Thus, the root length measure included both crop and weed roots. No measures of root length were made on the dairy farms.

3.4.b. Root Weights

Root dry weights of field or glasshouse trial samples were obtained after samples had been washed on a 2 mm sieve and as much remaining non-root material as possible removed using tweezers. Roots were then dried for 24 hours at 70°C.

When assessing the growth of plants from glasshouse trials it was often necessary to measure the total root dry weight of samples which were also to be subsampled and assessed for VAM colonisation. In these cases, when assessing VAM colonisation (§3.5.b), the length of the roots stained was measured using the line intersect method (§3.4.a) and this figure was converted to root dry weight using the root dry weight-length ratio. The root dry weight-length ratio was calculated on 10 subsamples of roots randomly selected from all the samples by measuring the root length of individual samples, before drying and weighing. The roots from each sample that were not stained were dried and weighed. The total dry weight of roots in each sample could then be calculated.

Root wet weight measures were also made in some instances. Roots were washed, blotted dry between folded sheets of paper towel and left to air dry for 10 minutes. They were then weighed and a subsample, later to be stained for VAM colonisation, was taken and stored in 80% ethanol. For consistent presentation of results and calculation of root-shoot ratios, wet weights were converted to dry weights by calculating a conversion factor on 10 subsamples randomly selected from all samples. Whilst less accurate than direct measurement of root dry weights, root wet weights were often used in assessing plants from glasshouse trials. By weighing the roots before they were subsampled for VAM assessment — which can not occur after roots have been dried — the lengthy process, described above, for calculating the total dry weight of samples which were subsampled for VAM assessment, could be avoided.

3.4.c. Root Nutrient Concentrations

The concentration of P and N in roots was measured using the method described above for shoots (§3.3.b). Care was taken to ensure that roots were as clean as possible, in order to avoid errors due to inclusion of soil in the analysis. All roots were washed a number of times in ethanol or water and all visible remaining soil removed using tweezers. Only clover roots were analysed in this manner as roots of grasses, such as wheat and rye grass, could not be adequately cleaned.

3.4.d. Level of *Rhizobium* Nodulation in Clover

Samples of clover roots were placed on a petri dish and root length calculated (§3.4.a). Samples were then scanned at x15 magnification and the total number of nodules counted. Results were expressed as the number of nodules each 100 mm or metre of

root length. Roots were returned to 80% ethanol and later stained for assessment of VAM colonisation (§3.5.a).

3.5. VAM Fungi

3.5.a. Staining Roots to Distinguish VAM Fungi

Clearing and staining roots to distinguish VAM fungi was based on the method of Grace and Stribley (1991). Roots were removed from the 80% ethanol in which they had been stored and any remaining soil removed by washing in 80-90% ethanol. To digest the nuclei and cytoplasm of host plant cells, 10% potassium hydroxide was added and roots heated at 87°C in a water bath for 30-45 minutes, depending on plant age and species. Roots were then rinsed three times in water and 2% hydrochloric acid added for 10-30 seconds. Stain, 1% aniline blue in water diluted to 0.05% in 70% glycerol, was then added and samples heated for 5-6 minutes in a water bath at 87°C. The stain was removed and 70% glycerol added. Roots were left for > 24 hours to allow the excess stain to leach into the glycerol, leaving blue-stained fungal tissue against a light green background of plant tissue.

3.5.b. Assessing the Level of Colonisation by VAM Fungi

The percentage of root length colonised by VAM fungi — VAM (%) — was calculated using the line-intersect method (Giovannetti and Mosse 1980). Stained root samples were cut into approximately 1 mm sections and spread over a square petri-dish inscribed with a 12.73 mm grid. Grid lines were scanned under a dissecting microscope. At each intersect between a root and a grid line, the section of root crossing the line was noted as being colonised, or not colonised, at the point of intersection. For each sample, > 100 intersects were scored. The number of intersects which were colonised was divided by the total number of intersects examined and the percentage of root length colonised calculated. All samples were randomly coded to ensure objective assessment.

3.5.c. Assessing Colonisation Intensity

On some root samples an assessment of the intensity of VAM colonisation was also made. At each intersection between a root and a gridline where VAM colonisation was present, colonisation intensity was classified as low, medium or high, as described below; see Evans and Miller (1988) and Daniels Hetrick *et al.* (1988).

Low:	From a single hypha or a number of small isolated areas of colonisation to less than half the root cortex colonised.
Medium:	Half to all of the root cortex filled with continuous colonisation of low to medium density.
High:	Dense colonisation of the entire root cortex.

The intensity measures were used to calculate an adjusted measure of VAM colonisation (VAM intensity) using the formula presented below which gave a score out of 400. This adjusted measure was considered to be a more accurate reflection of the volume of fungal material present in the roots than VAM (%).

$$\text{Colonisation level adjusted for intensity} = \text{Percentage of root length colonised at low intensity} + 2 \times \text{Percentage of root length colonised at medium intensity} + 4 \times \text{Percentage of root length colonised at high intensity}$$

3.5.d. Extraction of VAM Spores

Samples of soil were soaked for > 12 hours in anhydrous tetrasodium pyrophosphate ($Na_4P_2O_7$) to disperse soil particles and organic matter. As preliminary observations found many spores strongly adhering to pieces of organic matter, the solution was not simply decanted. Instead, all of the soil solution was vigorously stirred and poured through a set of 250, 150, 90 and 50 µm sieves. Material on the 250 µm and 50 µm sieves was discarded, as few spores were caught on these sieves. Material on the remaining two sieves was washed into a nematode counting dish and all spores counted at x 10 magnification under a dissecting microscope. This method involved a large proportion of the soil in each sample being examined under the microscope and thus each sample was relatively small; 1-2 g. Results were expressed as number of spores in a gram of dry soil.

3.6. Soil Measurements

Soil samples from crops were collected to 100 mm depth from the row and inter-row at each site and soil samples from pastures were collected to 100 mm depth at each sampling site. Unless noted in the methods section of a particular chapter, soil samples were collected and analysed separately for each of the 10-15 sites in a paddock. Soil samples from glasshouse trials were taken from the bulked soil used in the trial. All samples were stored at 4°C until processing, when they were air dried at ambient temperature before being crushed, passed through a 2 mm sieve and stored in a plastic container. Samples to be assessed for pH in a 1:5 water dilution, total N and extractable

P — measured either by the method of Colwell (1963) or Olsen *et al.* (1954) — were sent to the Soil Fertility Laboratory at Wesfarmers CSBP (Perth, Western Australia). To be consistent with the data provided by Derrick and Small, extractable P was measured using the Olsen method on the dairy farm soils and the Colwell method on the mixed farm soils.

Determination of pH was also carried out in the Soils Analysis Laboratory in the Forestry Department, Australian National University. Samples were air dried and sieved through a 2 mm sieve. Five gram subsamples were diluted in distilled water, shaken for 1 hour and left overnight before determination of pH (Rayment and Higginson 1992).

Measures of soil nutrient concentrations in 1993 crops (Chapter 5) were provided by Derrick (Derrick 1996). Soil cores, 100 mm diameter, were taken to 150 mm in the crop row and inter-row at each site and combined. The soil was air dried at ambient temperature, passed through a 2 mm sieve and stored in a plastic container. Subsamples were oven dried at 65°C for 12 hours. Total N and total P were determined by the automated spectophotometric method (Heffernan 1985b), extractable P by the Colwell method (Colwell 1963) and pH determined in a 1:5 water dilution (Rayment and Higginson 1992). Measures of soil nutrients on the dairy farms (Chapter 10) were provided by Small. Soil cores were taken to 100 mm from 20 sites in each of two paddocks on each farm and bulked. Samples were dried at 40°C and passed through a 2 mm sieve. Soils were analysed by the chemistry laboratory, ISIA, at Tatura for extractable P (Olsen *et al.* 1954), total P in the Olsen extract, Colwell P (Colwell 1963), total Kjeldahl N and pH in a 1:5 water suspension (Small *et al.* 1994b).

3.7. Glasshouse Trials

All glasshouse trials were carried out at the Plant Culture Facility, Australian National University. The trials were designed to produce results as relevant to field conditions as possible, whilst utilising the factors — such as uniform environmental conditions and production of non-VAM controls — which allow glasshouse trials to show relationships not able to be quantified, or even examined, under field conditions (§1.5).

All glasshouse trials used undiluted soil collected from the field. Weighing of individual pots to water exactly to field capacity was not possible due to the large number of pots in most trials. Consequently, pots were watered by hand when the soil appeared dry, until water began to flow from the bottom of the pots.

Gamma irradiated soil was treated at 25 kGy, as recommended by Thompson (1990), at the Australian Nuclear Science and Technology Organisation, Lucas Heights, Sydney. When such sterilised soil was used, filtrate was added to all pots to balance soil micro-organism populations between pots containing only sterilised soil and pots

which had received non-sterile inoculum. The filtrate consisted of the aqueous extract from 400 g of the soil used as inoculum, which had been passed through a 38 μm sieve to exclude VAM spores and made up to 5 l (Daniels Hetrick *et al.* 1988).

To ensure that plants were not affected by nutrient deficiencies other than P, basal nutrients, minus P, were applied in most of the glasshouse trials at the following rates (mg pot^{-1}): NH_4NO_3 120; K_2SO_4 65; $CaCl_2$ 37.5; $MgSO_4.3H_2O$ 16.7; iron chelate 4.6; H_3BO_3 2.15; $MnCl_2.4H_2O$ 1.36; $ZnSO_4.7H_2O$ 0.17; MoO_3 0.015; $CuSO_4.5H_2O$ 0.06. Other methods varied between trials and are detailed in the methods sections in Chapters 7 and 11.

3.8. Climate Data

All rainfall and temperature figures were supplied by the Bureau of Meteorology. Occasionally, when figures were missing for individual months, they were either obtained from local farmers who kept their own records or calculated from the figures recorded for surrounding weather stations. When rainfall was given for the crop growing season, this was calculated as the rainfall from the month of sowing until November, or the month in which the final sampling occurred.

3.9. Data Presentation and Statistical Analyses

Several aspects of project design were maintained in all analyses. Most field sampling occurred on adjacent conventional/alternative farm pairs and thus 'farm pair', as a nominal variable, was usually included in analyses. In practice, the pair variable was often important in interpreting results as it acted as a location effect, reflecting trends in underlying factors such as soil type and climate, not otherwise measured. 'Farm management strategy' was included in analyses when conclusions were being drawn on differences between conventional and alternative farms; data presented in graphs or tables is often grouped by management strategy. However, the differing farm management strategies were primarily included in this project as a means of providing replicated treatments varying in P inputs. Therefore, management strategy is often replaced in models with continuous measures of soil or plant P concentrations. Field data is often presented, particularly in graphs, with changes over 'time' being emphasised, either as changes over a cropping season or comparisons between seasons or years. A nominal 'block' variable was included in the design and analysis of all glasshouse trials in order to factor out spatial variations across the glasshouse.

Graphs were used to check for outliers in all data sets. Outliers were only removed if they appeared anomalous to the rest of the data set, that is, if they represented a very small proportion of the entire data set — at most, two data points —

and were exerting a strong influence on the analysis, resulting in otherwise strong trends being obscured.

All field data were initially examined graphically and results are presented in this manner where appropriate. All graphs of field data in which mean values are being compared include error bars, which represent the standard error of the mean (s.e.m.). The number of samples which were averaged to obtain the mean values is stated in the caption. Data presented in tables also have s.e.m.s provided.

Further statistical modelling was performed on some field data and all glasshouse trial data, using the SAS Institute software JMP® (version 3.1) to fit linear models; ANOVA, ANCOVA and regression. Details of the exact analyses performed are given in the methods section of the relevant chapter, however, in all cases the fit of the model and the residuals were examined and, if necessary, the data were transformed to achieve normality or homogeneity of variance. For instance, plant growth data often required log transformation ($\log_{10}$ was used in all cases). When transformations were preformed, this is noted where the results of the analysis are presented. Most field measures of VAM colonisation were in the form of percentages and while this might indicate transformation or logistic regression analysis, in practice, most values fell between 10% and 90% and the residuals did not deviate greatly from normality; thus analyses were performed on untransformed data.

The details of the statistical models described above are presented in tables which contain an indication of the power (F-ratio), significance (probability level, 'prob.') and the fit of the entire model (adjusted r^2 value, 'r^2'); the number of observations, 'n', is also provided. In all analyses, an F-ratio and probability level are given for all predictor variables. In addition, in regression analyses, the coefficient, 'coefficient' and its standard error 's.e.' are given for the model intercept and all predictor variables, along with a t-test and probability level for the intercept.

Stepwise regressions were also performed on some data sets to assess whether farms under different management strategies were functioning through the same biological relationships (see §2.3.c). Measures of biological activity, such as crop biomass and VAM colonisation, were first fitted with biological and soil factors, such as soil and plant nutrient concentrations. 'Location' was then added to take account of climatic or other variables that may have consistently varied between locations. Finally, 'farm management strategy' was added to ascertain whether variation in the data could be explained by factors which varied consistently with farm management strategy, but had not been explained by the variables already added. These analyses are presented in tables with the cumulative adjusted r^2 and change in adjusted r^2 provided for each additional variable.

When constructing statistical models which used field data, only those predictor variables which had a significant effect in the model were included. For glasshouse

trials, all factors included in the original design, including block, were included in the model, even if they were found not to be significant.

If the variables in the model were continuous, as was usually the case for field data, the most significant relationships between the dependent and predictor variables are often also presented graphically as simple regressions using the raw data. If the major predictor variables were nominal, as was the case for all glasshouse trial results, the data are presented as bar graphs and all significant interaction terms are also presented graphically. When presenting data from glasshouse experiments which were designed to test specific questions, the outcomes are presented as the estimated least squares means adjusted for the other factors in the model, with the least significant difference (LSD) for the whole model at the 5% level included.

3.10. Collaboration

Some of the research reported in Chapters 5 and 10 was conducted in collaboration with other researchers. The dairy farms had already been selected and studied for two years by Mr Doug Small (Victorian Department of Agriculture, Institute for Sustainable Irrigated Agriculture, Kyabram). Much of the soil and plant nutrient data presented from these farms (Chapter 10) was provided by Small and can also be found in the final project report prepared by Small and co-workers (Small *et al.* 1994a). Similarly, the 1993 sampling of mixed farms (Chapter 5) was conducted in collaboration with Dr Jim Derrick, then a PhD student in the Geography Department at the Australian National University. Much of the soil and plant nutrient data and crop growth data in Chapter 5 was supplied by Derrick and detailed information about the two farms at Yenda and the organic and conventional farms at Ardlethan can be found in his PhD thesis (Derrick 1996).

Whenever data is presented which has been supplied by other researchers, this fact is acknowledged in the methods section of the relevant chapter, as well as in the caption of any figures or tables which use the data. Unfortunately, data obtained from other sources occasionally consisted only of mean values and did not contain any indication of the level of variation around the mean. Details of where data have been published in greater detail are provided when possible.

Part C

Mixed Cereal-Livestock Farms

Chapter Four

The Mixed Farms Studied in this Project

This chapter presents a general introduction to the southern wheatbelt of NSW and the mixed farms which were studied in this project. Other studies on these farms which have links with this project are listed. Rainfall figures for the period over which field sampling occurred, 1993-1995, are given to allow comparisons between years. Owing to the small number of farms examined, in contrast to the dairy farms introduced in Chapter 9, information on farm characteristics and management is given for individual farms. A general summary of differences between the conventional and alternative farms is also provided.

4.1. General Characteristics of the Study Sites

The mixed farms sampled during this project are located in the southern wheatbelt of Australia at Yenda, Ardlethan and Cootamundra (Fig. 4.1). The southern wheatbelt consists of temperate semi-arid slopes and plains and contains a large proportion of Australia's arable land. It spreads from northern NSW, across central Victoria and SE-South Australia, and also includes a large area of SW-Western Australia (Fig. 4.1). The major climatic constraint to agricultural production in this region is rainfall which is aseasonal, but, when coupled with hot summers results in a winter-spring growing season due to higher soil moisture over this period. Elevation and climatic characteristics for each location are listed in Table 4.1.

Table 4.1. Elevation, rainfall (89 year average), and winter and summer minimum and maximum daily temperatures (28 year average) at Yenda, Ardlethan and Cootamundra (data provided by the Bureau of Meteorology).

	Yenda	Ardlethan	Cootamundra
Elevation (m)	129	204	318
Average annual rainfall (mm)	403	490	621
Average mean minimum and maximum daily temperatures in winter (°C)	3.7 - 15.4	3.2 - 14.8	2.5 - 13.6
Average mean minimum and maximum daily temperatures in summer (°C)	16.1 - 31.6	15.5 - 31.3	15.1 - 30.8

The areas sampled during this project were first settled in the 1850s, with most land taken up in large stations by squatters and used for grazing. After the Land Act of 1884, the large stations were subdivided and by the early 1900s most land had been taken up by selectors who began cropping wheat and established commodity rotations (Webster 1956). By the 1930s, use of superphosphate had become widespread and inputs of fertilisers and chemicals have continued to increase from this time.

Currently, most farms within this region are mixed and are based on the production of winter crops of cereals rotated with mixed pastures which grow on an annual cycle with above-ground foliage tending to die back over summer. The pastures contain grasses, legumes and broad leaved plants including subterranean clover (*Trifolium subterraneum* L.), annual rye grass (*Lolium rigidum* Gaud.), silver grass (*Vulpia bromoides* (L.) S. F. Gray.), capeweed (*Arctotheca calendula* (L.) Levyns) and Paterson's Curse (*Echium plantagineum* L.). There is generally little direct management of the pasture, other than through the application of fertiliser at

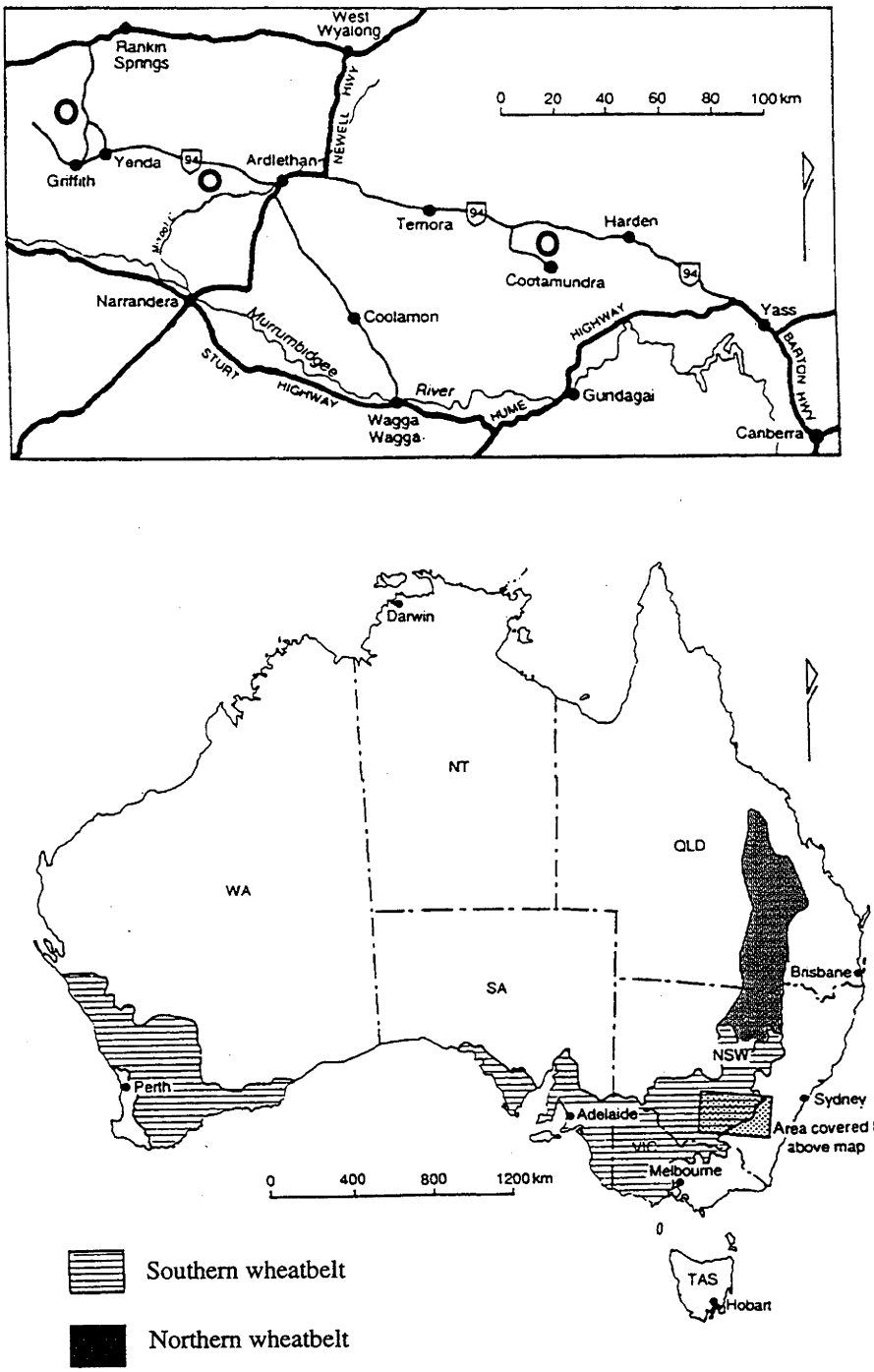


Figure 4.1. Location of mixed farms. Sampling sites at Yenda, Ardlethan, and Cootamundra indicated by hollow circles on upper map.

Plate 4.1. A paddock about to enter a first year of cropping under summer fallow on the organic farm at Ardlethan.

Plate 4.2. A first year wheat crop heavily undersown with pasture species on the biodynamic farm at Cootamundra.

establishment and the manipulation of stocking rates. The pastures are grazed by sheep, used for production of wool and meat, and beef cattle.

After 3-8 years the pasture is killed by herbicides or tillage in October or November. The soil is then left bare for two to eight months over summer — a summer fallow (Plate 4.1) — to conserve water and control weeds, before further tillage and sowing of a first year crop the following autumn in May or June. The crops grown are predominantly cereals such as wheat *(Triticum aestivum* L.), barley (*Hordeum* sp.) and oats (*Avena sativa* L.), as well as grain legumes such as lupins (*Lupinus* spp.) and oilseed crops including canola (*Brassica napus* L.). After harvest in late spring (November or December), the stubble and any pasture plants which recolonised are grazed by stock over summer, before the paddock is tilled and a second year crop sown the following autumn. After 1-3 years of cropping, pasture is allowed to re-establish from the residual seed bank, perhaps supplemented by sowing of some favoured species.

The soils on the study sites are red earths, which are typical of the soils in much of the southern wheatbelt. Their distinguishing characteristics are massive, predominantly sandy texture, porous and earthy soil materials, red-brown to red colour, weak profile differentiation with gradual or diffuse horizon boundaries except for the darker A_1 horizon, and acid to mildly alkaline reaction (Stace *et al.* 1968). Their low water holding capacity is a limitation for dryland farming. Soil nutrient concentrations are generally low and heavy fertilisation with P and N is needed for maximum yields (Stace *et al.* 1968). Soil organic matter levels are also low and are generally declining, resulting in soil structural problems (Davidson 1986). Appendix 1 contains profile descriptions from the organic farm at Ardlethan.

4.2. Data Collection and Presentation

4.2.a. The Paired Farms Sampled at each Location

With the exception of Cootamundra in 1993, when no suitable conventional neighbour was available, a farm under alternative management was paired with a conventional neighbour at each location (Table 4.2). Three conventional farms (I, II, and III) were sampled successively at Ardlethan, as two farms were sold and purchased by absentee landlords who did not continue to manage the farms as typical conventional mixed operations. The conventional I farm at Ardlethan was not sampled during this project, however it is referred to occasionally, as it was sampled in 1992 during a preliminary study by Ryan (1992) and fertiliser trials, described in Dann *et al.* (1996), were conducted on the farm in 1991 and 1992. The conventional I farm was sold after harvest in 1992 and subsequently leased to the neighbouring organic farmer who, in 1993, grew a wheat crop under organic management. This crop was sampled during

this project and is referred to as the 'conversion' crop. The mixed farms are always identified by location and management strategy, for instance 'Ardlethan organic'.

Table 4.2. The paired mixed farms sampled during this project.

Year	Yenda	Ardlethan	Cootamundra	Thesis chapter
1993	conventional organic	conventional II conversion organic	biodynamic	Chapter 5 - crops
1994		conventional III organic	conventional biodynamic	Chapter 8 - drought
1995		conventional III organic	conventional biodynamic	"
1996		conventional III organic	conventional biodynamic	Chapter 6 - pastures

The farms at Ardlethan were the main focus of research. The organic farm at Ardlethan had been under organic management since 1962, a substantially longer period of time than the other alternative farms and it was therefore considered to be the farm most likely to have adapted in response to organic management. The farms at Ardlethan were also being studied by other researchers, providing opportunity for collaboration.

4.2.b. Use of Data from Other Studies

Results from a preliminary study of the factors controlling VAM colonisation in wheat in 1992 on the conventional I and organic farms at Ardlethan (Ryan 1992; Ryan *et al.* 1994) and the results from fertiliser trials also conducted on these two farms in 1991 and 1992 (Dann *et al.* 1996), are referred to when appropriate. The farms at Ardlethan were studied from 1991 to 1993, and the farms at Yenda in 1993, by Derrick as part of a PhD project. Fieldwork during 1993 was conducted in conjunction with Derrick and data summarised in Derrick (1996) is presented when necessary.

4.2.c. Rainfall Data

Monthly rainfall figures, provided by the Bureau of Meteorology, are provided at the beginning of each chapter for the period over which sampling occurred. To allow comparisons to be made between the different years, Figure 4.2 contains the monthly rainfall totals at Ardlethan and Cootamundra (sampling occurred at Yenda only during 1994) from 1993 to 1995, along with the long term averages. Rainfall during 1993 and 1995 at both locations was above or close to the average. However, in 1994 there was a

severe drought, one of the worst this century. Thus 1994 provided an opportunity to examine the effects of drought on VAM colonisation and crop growth.

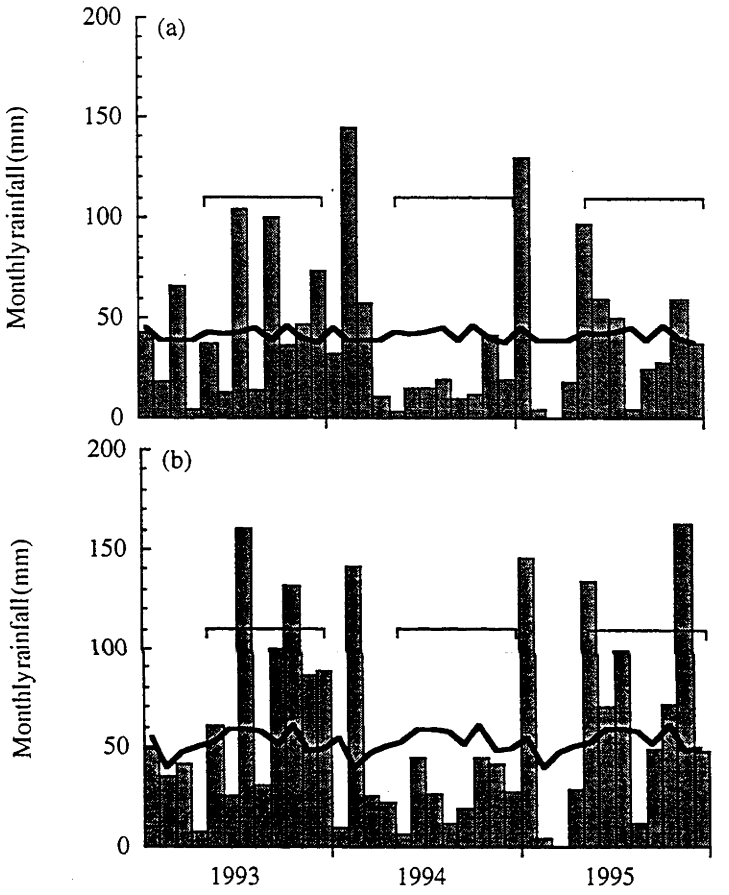


Figure 4.2. Monthly rainfall from 1993 to 1995 (bars) and the long term average monthly rainfall (line) at a) Ardlethan and b) Cootamundra. Crop growing seasons are indicated for each year.

4.3. Management Characteristics of Farms

Selected characteristics of the farms sampled during this project are listed in Table 4.3. Farm management varied due to a range of factors including farm management strategy — conventional or alternative — farm size, the environmental conditions present on each farm, and the specific goals and philosophies of the individual farmers. However, there were a number of consistent differences between the conventional and alternative farms (see also §2.3).

Table 4.3. Characteristics and management of mixed cereal-livestock farms.

Location	Yenda		Ardlethan				Cootamundra	
System	Conventional	Organic	Conventional I	Conventional II	Conventional III	Organic	Conventional	Biodynamic
Size (ha)	445	3933/3000 arable	400	400	365	1080/750 arable	288	427
Typical rotation	1 lupins, 1 oats, 1 wheat, 1 pasture	1 wheat/barley, 1 oats, 2 pasture	2 wheat, 1 barley, 3 pasture	2 wheat, 1 barley, 3 pasture	1 wheat, 2 pasture	1 wheat, 1 wheat/oats/rye, 5 pasture	1 oats, 1 wheat, 1 canola, 1 wheat, 1 lupins, 1 wheat, 1 triticale, 4 pasture	no fixed rotation, crop one year then return to pasture
First tillage	September	February	December	December	January	October	October	February (October if summer cropping)
Cultivations before sowing	three	four	three	three	three	three	two	four
Fertilisers	super-phosphate	mineral fertilisers	diammonium phosphate	diammonium phosphate	diammonium phosphate	reactive rock phosphate (paddocks limed once)	diammonium phosphate	reactive rock phosphate (most paddocks limed in 1995)
Biocides	yes	no	yes	yes	yes	no	yes	no
Stubble	burnt	retained	burnt	burnt	burnt	retained	burnt	retained
Stock	-	1700 merino ewes	500 cross-bred merino & border leister ewes	500 cross-bred merino & border leister ewes	500 ewes	1000 merino ewes, 90 cattle	700 crossbred ewes	1300 crossbred ewes, 40 cattle, 20 free-range sows
Other	owned by a large chicken farming company	organic since 1986	sold end of 1992	sold end of 1993	-	grain processed on farm and sold as flour, organic since 1962	lease expired at end of 1995	crops undersown with pasture species, biodynamic since 1987

The alternative farms were larger than the conventional farms, allowing a greater proportion of the rotation to consist of annual pasture which has lower economic returns than cropping. Alternative farms also tended to use a longer fallow (Plate 4.1) as the first cultivation was earlier in spring or summer, resulting in the ground being left bare for a longer period before the crop was sown in the following autumn. This longer fallow was designed to aid with weed control, as well as to conserve soil moisture and allow N mineralisation. However, overall, there was little difference in the number of cultivations associated with each crop on the conventional and alternative farms. While the conventional farmers used herbicides and fertilisers containing relatively soluble nutrients, the alternative farmers applied no biocides and used relatively insoluble mineral fertilisers, often applied at very low rates. Crop stubble on the conventional farms was routinely burned, unless a paddock was to be returned to pasture, while all alternative farmers retained stubble. The alternative farms also differed commercially in supplying a relatively small and specialised market, with sales directed towards retail outlets, as opposed to using traditional marketing systems. Alternative farmers often added value to their produce on the farm through processes such as flour milling.

The biodynamic farm at Cootamundra differed from the other alternative farms in a number of ways. There was no fixed rotation; when the farmer considered that pasture in a paddock required rejuvenating, the paddock was cropped for one season before being returned to pasture. The primary aim of cropping was improvement of pasture composition and growth and, therefore, crops were always heavily undersown with a variety of pasture legume and grass species (Plate 4.2), including strawberry clover (*Trifolium fradiferum* L.), white clover (*T. repens* L.), phalaris (*Phalaris aquatica* L.) and cocksfoot (*Dactylis glomerata* L.). A large number of crops were grown on the farm including wheat, barley, triticale (*Triticum* x *Secale*), lupins, and summer forage sorghum (*Sorghum* sp.).

The higher rainfall at Cootamundra (Table 4.1) made it possible to grow an additional crop over summer. During this study, most crops on the biodynamic farm were preceded by a crop of summer forage sorghum. The summer crop was grazed *in situ* and the farmer considered that the organic matter remaining from the crop and the animal manure produced during its grazing provided a source of available P for the following winter crop. To minimise loss of nutrients from the farm, grain was not sold, instead being used to feed stock including free-range pigs, sheep, goats, and cattle.

Additional information on these farms can be found in a number of publications: management of the organic farms at Yenda and Ardlethan (Wynen 1992); crop growth and nutrition at Yenda and Ardlethan (Derrick 1996); energy flows at Ardlethan (Derrick 1994); soil structure and VAM fungal hyphae at Ardlethan (Gatehouse 1995); levels of VAM fungi at Ardlethan (Ryan 1992; Ryan and Ash 1996; Ryan *et al.* 1994; Ryan and Dumaresq 1994); cellulolytic fungi at Ardlethan (Ryan 1992), response of

conventional and organic crops at Ardlethan to superphosphate and rock phosphate additions (Dann *et al.* 1996), and the influence of seed P concentration and content on growth of wheat seedlings in soil from the organic farm at Ardlethan (Derrick and Ryan 1998).

Chapter Five

VAM Colonisation Levels, Soil and Crop Nutrient Concentrations, Crop Biomass and Weed Abundance in First Year Cereal Crops

A field survey was conducted of first year cereal crops, primarily wheat crops, on six dryland mixed farms during the winter 1993 cropping season. Individual farm locations and management characteristics were described in Chapter 4. Results are grouped into three main areas: 1) the extent of VAM colonisation in crops; 2) the growth and nutrition of crops; and 3) the relationships between VAM colonisation levels, soil and crop nutrient concentrations, crop biomass and weed abundance. The field survey was conducted in conjunction with Derrick and much of the data presented on soil nutrient concentrations, crop nutrient concentrations, weed biomass and crop biomass were provided by Derrick and are summarised his PhD thesis (Derrick 1996).

5.1. Aims

The aims of the research reported in this chapter were to investigate the following questions in regard to first year cereal crops on six mixed farms in 1993.

- What were the major factors influencing the percentage of root length colonised by VAM fungi — VAM (%) — in the crops?

- Did alternative measures of VAM presence — colonisation intensity and length of colonised root — provide a more useful measure of VAM colonisation levels than the commonly used measure, VAM (%)?

- What were the major factors influencing crop biomass, crop root length, crop P concentration and crop P content?

    - In particular, did VAM (%) influence any of the above parameters?

- Were the relationships between crop biomass, soil and crop nutrient concentrations, and VAM colonisation levels similar on the conventional and alternative farms?

5.2. Methods

Over the 1993 cropping season six mixed farms were sampled four times: an organic and conventional farm pair at Yenda; an organic, conventional (II) and farm under conversion to organic management at Ardlethan; and a biodynamic farm at Cootamundra for which no conventional neighbour could be arranged (Table 4.2). A first year wheat crop — first crop grown in a paddock after a number of years of pasture — was sampled on each farm. A barley crop on each farm at Yenda was also sampled.

Management details of these crops are given in Table 5.1. Tillage levels and sowing dates were similar for crops at each location, however crop variety differed between farms. The crops on the alternative farms had less nutrients applied in fertilisers and those nutrients which were applied were in less soluble forms. Only the conventional crops received post-sowing herbicides. The biodynamic crop at Cootamundra differed from the other crops in being sown with twice the distance between wheat rows (350 mm) and being heavily undersown with pasture species (Plate 4.1).

Crops were sampled on 17 July (seedling), 4 September (tillering), 17 October (anthesis), and at harvest on 16 November (Yenda), 25 November (Ardlethan) and 18 December (Cootamundra). At each sampling, 15 sites were selected in each crop following the procedure described in Chapter 3 (§3.2). One plant from each site was removed and shoot dry weight, VAM (%), and VAM intensity measured as described in Chapter 3 (§3.3 and §3.5). Intensity results are presented both as the proportion of colonisation in each of the three intensity categories and as an adjusted measure of VAM colonisation (VAM intensity) which gave a score out of 400 (§3.5.c). Due to

Table 5.1. Management details of first year cereal crops sampled in 1993. Active ingredients of chemicals are given in Appendix 2.

Location	Farm management strategy	Years in pasture before crop	Pre-sowing	Crop	Date of sowing	Fertiliser (kg ha^{-1})	Nutrients Applied (kg ha^{-1}) P	Others	Other
Yenda	conventional	4	summer lucerne, 3 cultivations	wheat cv. Dollarbird	18 June	24:14 Pivot 106.4	14.9	25.5 N	-
		3	3 cultivations, first in September	barley cv. Schooner	18 June	24:14 Pivot 106.4	14.9	25.5 N	post-sowing: Treflan 0.7 L ha^{-1} Avadex 1.5 L ha^{-1}
	organic	3	4 cultivations, first in February	wheat cv. Janz	23 June	Vicmill COF 39.2	1.2	5.1 Ca, 4.7 S, 1.2 N, 1.2 K	-
		5	3 cultivations, first in February	barley cv. Schooner	23 June	Vicmill B2 50.4	1.8	10.7 Ca, 8.6 S, 1.5 K, 1.0 Mg	-
Ardlethan	conventional	4	3 cultivations, first in December	wheat cv. Janz	12 June	diammonium phosphate 79	15.8	14.2 N	post-sowing: Hoegrass 1 L ha^{-1} Jaguar 0.5 L ha^{-1}
	conversion	3	3 cultivations, first in October	wheat cv. Banks	12 June	Moroccan reactive phosphate rock 125.3	16.4	44.0 Ca, 1.6 S	-
	organic	6	3 cultivations, first in October	wheat cv. Banks	12 June	Moroccan reactive phosphate rock 125.3	16.4	44.0 Ca, 1.6 S	-
Cootamundra	biodynamic	5	summer forage sorghum, 4 cultivations	wheat cv. Vulcan	6 June	Quinphos reactive phosphate rock 44.8	5.7	14.8 Ca	Undersown with pasture species Crop rows double spaced

problems which occurred with the staining of the tillering samples, VAM colonisation levels were assessed on the individual plants from only the conversion paddock at Ardlethan and the biodynamic farm at Cootamundra. Thus, unless stated otherwise, VAM colonisation levels from the roots sampled in soil cores (see below) are used for tillering. Up to five plants from each of the dominant weed species present on the farms were assessed for VAM colonisation, however, due to the limited nature of this data, these results were not analysed in detail, and are contained in Appendix 3.

Additional measurements were made on the six first year wheat crops. Soil cores were taken from the row and inter-row at 10 sites in each paddock at tillering and five sites in each paddock at anthesis. At tillering the top 50 mm of soil was sampled, while at anthesis, cores were taken from the 0-50, 50-100 and 100-150 mm depths. A measure of total active root length (crop plus weeds) was obtained as described in section 3.4.a and, using an estimate of the root dry weight-length ratio, root biomass was calculated. Root-shoot ratios at anthesis were calculated using the biomass of crop and weeds in each paddock (total biomass), as provided by Derrick (1996). In doing so, it was assumed that 60% of root biomass was in the top 150 mm of soil (see Hamblin *et al.* 1990; Pearson *et al.* 1991). Roots from soil cores taken at tillering were stained and VAM (%) assessed. These figures were used, along with the root length data obtained from the soil cores, to calculate the length of root colonised by VAM fungi in the top 50 mm of soil. At tillering the two youngest fully-emerged leaves on the wheat plants sampled from sites 1-10 in each paddock were analysed for P and N concentration (leaf P and N), as described in section 3.3.b.

Additional measurements of crop biomass and soil and crop nutrient concentrations at 15 sites in each of the six wheat crops were made by Derrick (1996). Crop biomass was measured at all four sampling dates and weed biomass at the later three sampling dates, as described in section 3.3.a. A simple assessment of the weed species present in each paddock was made after walking a transect across the paddock. Again, due to the limited nature of the weed data, these results are not analysed in detail, but are presented in Appendix 4. Crop yield was estimated at each site as described in section 3.3a. At all sampling dates, shoot material at each site was analysed for P and N concentration (crop shoot P and N) (§3.3.b). Soil extractable P (Colwell) was measured at each sampling date, while total N, total P and pH were measured only at tillering (§3.6).

Rainfall data were provided by the Bureau of Meteorology with the exception of the 1993 rainfall at Yenda which was provided by Richard Billings of "Wia-Wera", Yenda. For the purposes of modelling, rainfall was calculated as the total rainfall in the four months preceding sampling at that location. This was a simplistic approach, however as sampling of all farms occurred simultaneously it was considered to be adequate. Results are analysed and presented as described in section 3.9. In sections

5.3.a-d results are presented graphically, or in tables, as mean values from each farm, with standard errors given whenever possible. In section 5.3.e the relationships between the variables presented in sections 5.3.a-d are explored statistically with particular emphasis on investigating both which factors were most strongly influencing VAM colonisation levels and crop growth, and whether the relationships between various soil, plant and VAM measures were similar on the conventional and alternative farms.

5.3. Results

5.3.a. Rainfall

Rainfall was 30-60% above average at all locations (Table 5.2). Total and growing season (May to November) long term averages are 410 and 271 mm at Yenda, 487 and 294 mm at Ardlethan and 625 and 436 mm at Cootamundra (see also Fig. 4.2). Rainfall increased from Yenda to Cootamundra, that is, as farms were located further east; this is the expected trend (Table 4.1).

Table 5.2. Rainfall (mm) at Yenda, Ardlethan and Cootamundra in 1993. Monthly totals, total for 1993 and total for May to November; the growing season.

	J	F	M	A	M	J	J	A	S	O	N	D	Total	M-N
Yenda	62	21	50	1	28	12	74	19	84	117	28	58	553	362
Ardlethan	42	18	66	4	38	12	92	26	87	123	22	71	602	471
Cootamundra	49	35	41	8	60	26	161	31	100	132	86	88	817	685

5.3.b. Soil Nutrient Concentrations

Results from soil analyses are contained in Table 5.3.

Table 5.3. Colwell extractable P, total P, total N and pH of soil under first year conventional (Con.), organic, conversion and biodynamic wheat crops sampled in 1993 at tillering (Derrick 1996); mean *(s.e.m.)*, n=15.

	Yenda		Ardlethan			Cootamundra
	Con.	Organic	Con.	Conversion	Organic	Biodynamic
Extractable P ($\mu g\ g^{-1}$)	29 *(3)*	10 *(0.5)*	24 *(2)*	21 *(2)*	10 *(0.5)*	20 *(2)*
Total P ($\mu g\ g^{-1}$)	347 *(25)*	322 *(6)*	348 *(5)*	383 *(9)*	342 *(7)*	504 *(13)*
Total N ($\mu g\ g^{-1}$)	702 *(21)*	749 *(44)*	975 *(28)*	1031 *(32)*	1071 *(43)*	1738 *(87)*
pH	6.1	6.0	5.2	5.4	6.3	5.7

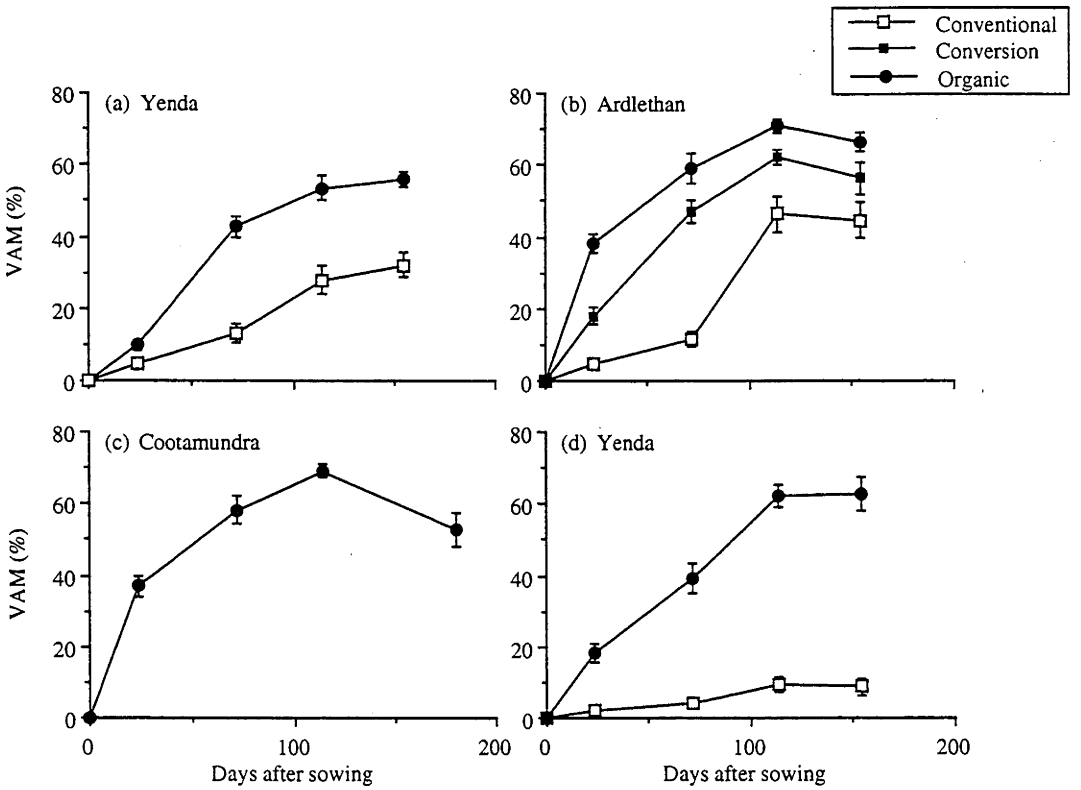


Figure 5.1. Percentage of root length colonised by VAM fungi over the 1993 wheat season in a) wheat at Yenda, b) wheat at Ardlethan, c) wheat at Cootamundra and d) barley at Yenda; mean ± s.e.m., n=15.

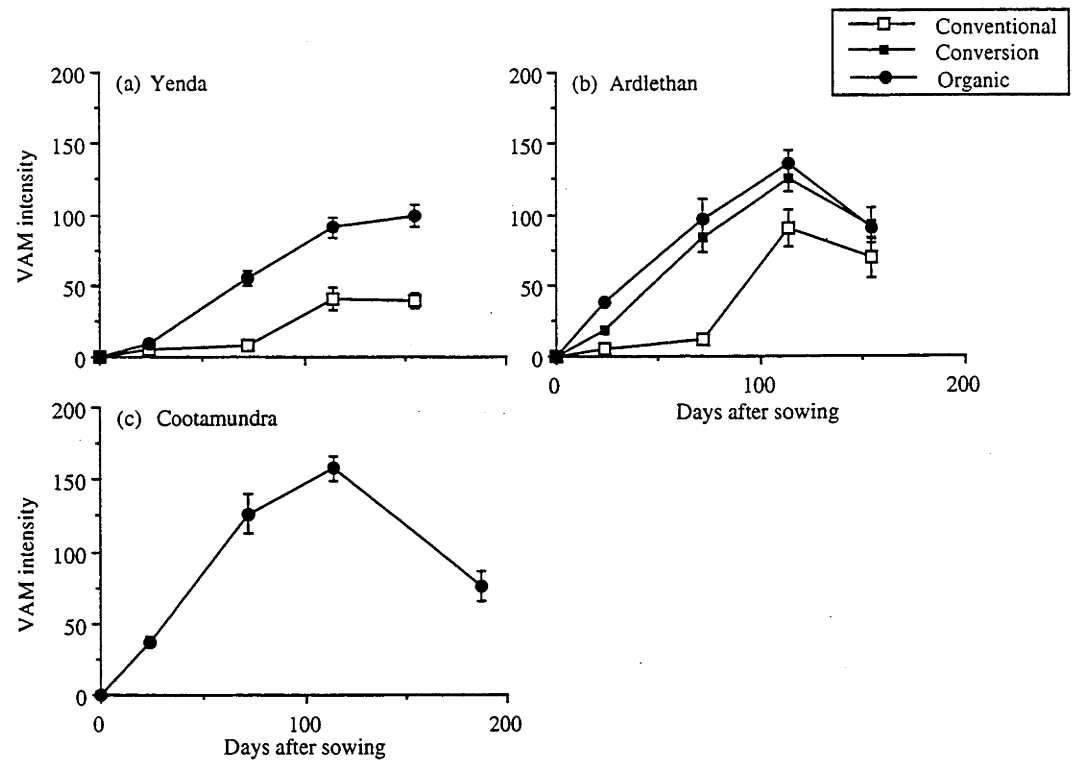


Figure 5.2. VAM intensity over the 1993 wheat season in wheat at a) Yenda, b) Ardlethan and c) Cootamundra; mean ± s.e.m., n=15.

Extractable P was 10 μg g^{-1} on the organic farms and 20-30 μg g^{-1} on the conversion, conventional and biodynamic farms. Total P was similar on the farms at Yenda and Ardlethan, but was considerably higher on the biodynamic farm at Cootamundra. Total soil N increased from Yenda to Ardlethan to Cootamundra. The pH was similar on the farms at Yenda, while at Ardlethan pH was higher on the organic farm.

5.3.c. VAM Colonisation Levels and VAM Intensity

The percentages of root length colonised by VAM fungi throughout the 1993 cropping season are presented in Figure 5.1. Throughout the season at all locations, colonisation levels on the conventional farms were lower than on the alternative farms in both wheat and barley. The conversion crop at Ardlethan had a colonisation curve intermediate to that of the conventional and organic crops and, in comparison to the organic crop, 25% less root length was colonised by tillering. VAM colonisation of the biodynamic crop at Cootamundra was virtually identical throughout the season to that of the organic crop at Ardlethan.

VAM intensity (Fig. 5.2) exhibited a generally similar pattern to VAM (%) (Fig. 5.1). However, the VAM intensity measure did result in the differences between the alternative and conventional farms being magnified; especially at the beginning of the season. The VAM intensity measures also showed the conversion crop at Ardlethan being more similar to its organic neighbour and a greater decrease in colonisation after anthesis at Ardlethan and Cootamundra.

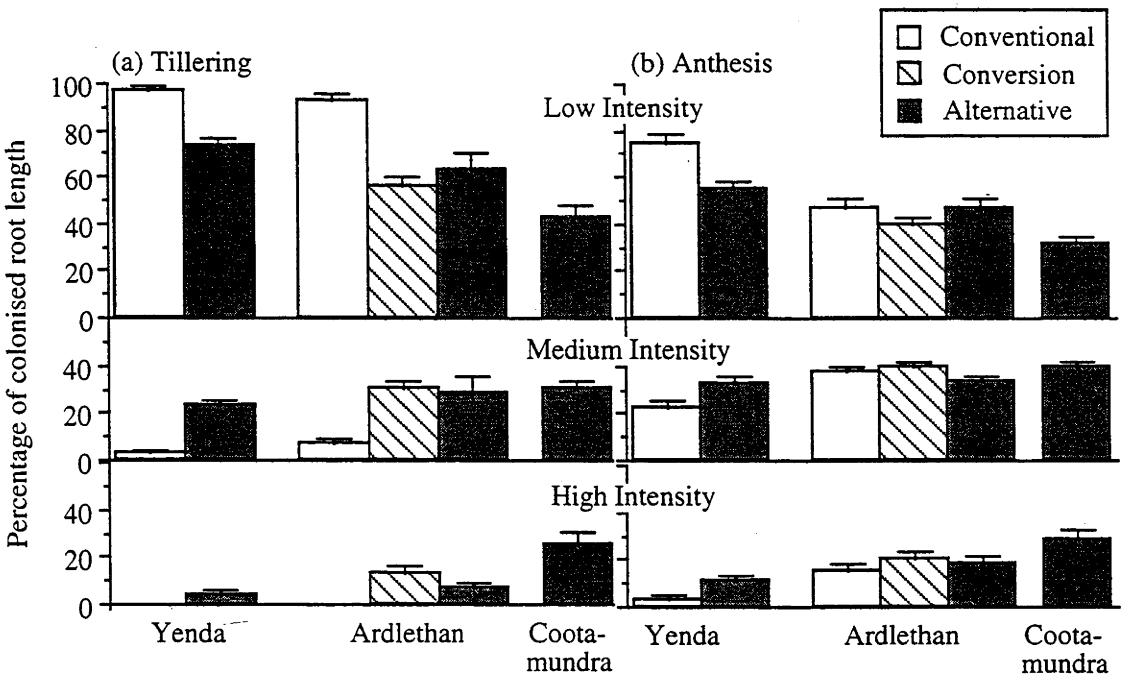


Figure 5.3. Percentage of colonised wheat root length in each of the three intensity categories at a) tillering and b) anthesis on the farms at Yenda, Ardlethan and Cootamundra; mean ± s.e.m, n=15. Low Intensity; less than half the root cortex colonised. Medium Intensity; half to all the root cortex filled with continuous colonisation of low to medium density. High Intensity; dense colonisation of the entire root cortex.

The percentages of the total colonised root length which was fell into the three intensity categories 'low', 'medium' and 'high' on each farm at tillering and anthesis are shown in Figure 5.3. At tillering, colonisation in the conventional crops was less intense than colonisation in the alternative crops. The two conventional crops had < 10% of total colonisation in the two higher intensity categories, while the alternative crops had 27-57% of colonisation in these categories. The biodynamic farm at Cootamundra had particularly intense colonisation. By anthesis the differences had lessened, however there was still a tendency for colonisation to be more intense in the alternative crops, particularly the biodynamic crop. At both tillering and anthesis the conventional crop at Yenda had a particularly low proportion of colonisation in the two higher intensity categories.

At tillering, VAM (%) was also measured in root samples obtained from soil cores taken on the crop rows and inter-rows (Fig. 5.4). The level of colonisation by VAM fungi differed greatly between the row and inter-row only on the conventional farms, where VAM (%) was higher on the inter-row. On the alternative farms there was a tendency for VAM (%) to be lower on the inter-rows.

The length of root colonised by VAM fungi under each crop is shown in Figure 5.5. At each location, at both tillering and anthesis, colonisation in the alternative crops was 1.8-4 times greater than in the conventional crops; a magnification of the results shown in Figure 5.1. The conversion crop at Ardlethan was intermediate to the conventional and organic crops.

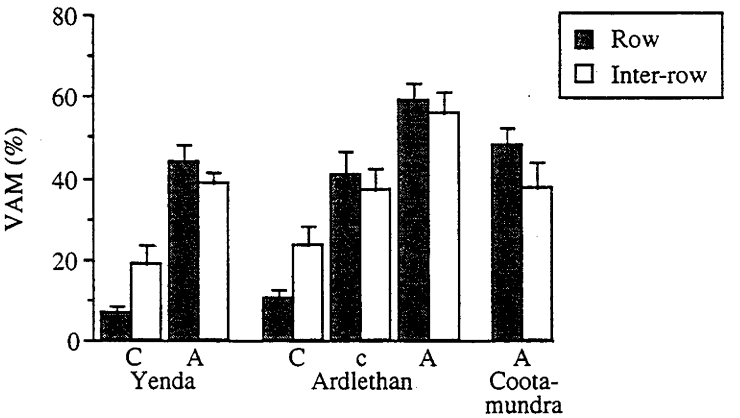


Figure 5.4. Percentage of wheat root length colonised by VAM fungi in the top 50 mm of soil on the crop rows and inter-rows on the conventional (C), conversion (c) and alternative (A) farms at Yenda, Ardlethan and Cootamundra at tillering in 1993; mean ± s.e.m., n=10.

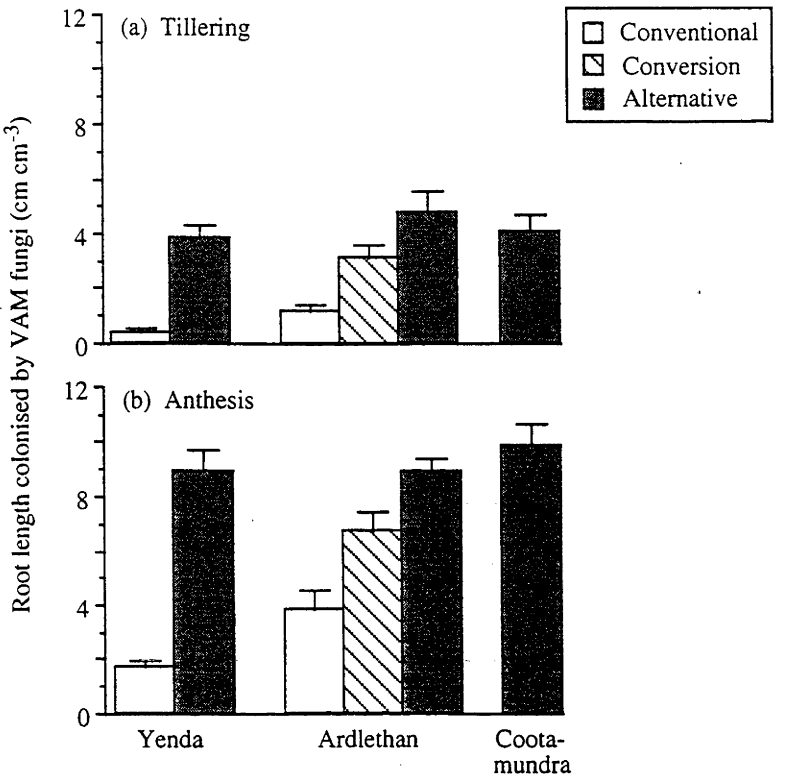


Figure 5.5. Root length colonised by VAM fungi in the top 50 mm of soil in the conventional, conversion and alternative wheat crops at Yenda, Ardlethan and Cootamundra, a) at tillering and b) at anthesis in 1993; mean ± s.e.m., n=10 at tillering, n=5 at anthesis.

5.3.d. Crop Growth, Yield and Nutrition

Crop biomass over the 1993 season and weed biomass between tillering and harvest are shown in Figures 5.6 and 5.7 for the six first year wheat crops. The percentage of total biomass at anthesis which consisted of weeds (all species other than wheat) and final grain yield are presented in Table 5.4. An estimate of the weed species present in each crop at anthesis and the frequency of their occurrence is contained in Appendix 4.

Table 5.4. The percentage of anthesis biomass on the conventional (Con.) organic, conversion (Conver.) and biodynamic farms which consisted of weeds and the final grain yield; mean *(s.e.m.)*, n=15. Data from Derrick (1996).

	Yenda		Ardlethan			Cootamundra
	Con.	Organic	Con.	Conver.	Organic	Biodynamic
Weeds (% total biomass)	6	72	0	29	9	29
Grain yield (t ha^{-1})	5.5 *(0.4)*	0.9 *(0.2)*	6.5 *(0.3)*	3.1 *(0.4)*	3.9 *(0.3)*	2.4 *(0.3)*

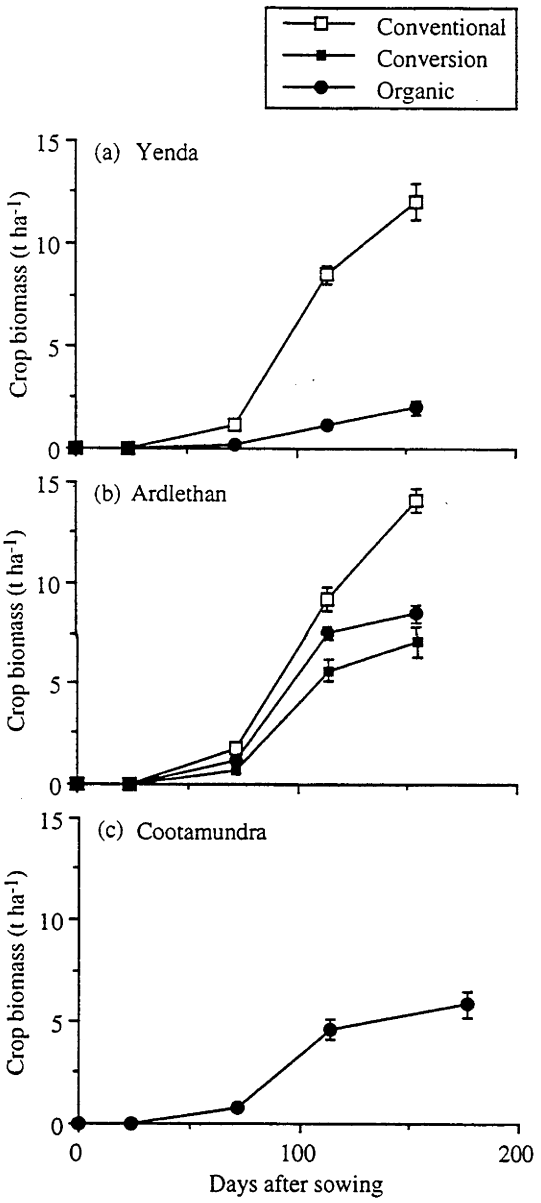


Figure 5.6. Wheat biomass over the 1993 season at a) Yenda, b) Ardlethan and c) Cootamundra; mean ± s.e.m, n=15. Data from Derrick (1996).

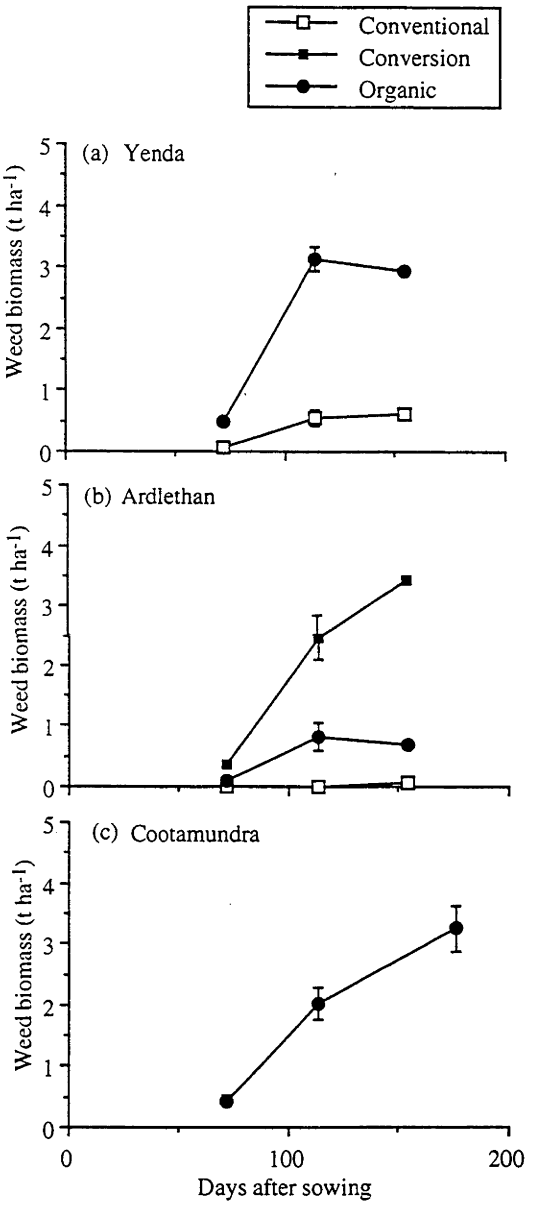


Figure 5.7. Weed biomass over the 1993 season at a) Yenda, b) Ardlethan and c) Cootamundra; mean ± s.e.m, n=15. Note the different scale to Figure 5.6. Data from Derrick (1996).

Crop biomass throughout the season and final grain yield were highest in the two conventional crops. Crop growth and yield were fairly similar for the alternative crops at Ardlethan and Cootamundra, but were considerably lower for the organic crop at Yenda. The conversion crop yielded slightly less than the organic crop at Ardlethan. Weed levels were negligible on the conventional farms and low on the organic farm at Ardlethan. The conversion crop at Ardlethan and the biodynamic crop at Cootamundra had a moderate level of weeds, while the organic crop at Yenda had an extremely high occurrence of weeds. However the biodynamic wheat was sown with rows twice as wide as the other crops and was deliberately undersown with pasture species (Table 5.1).

Root length at anthesis — crop and weed roots combined — was measured to 150 mm (Fig. 5.8). The alternative crops had a greater root length than the conventional crops in the top 50 mm of soil. The organic crop at Yenda and the biodynamic crop at Cootamundra maintained this difference at the 50-100 and 100-150 mm depths.

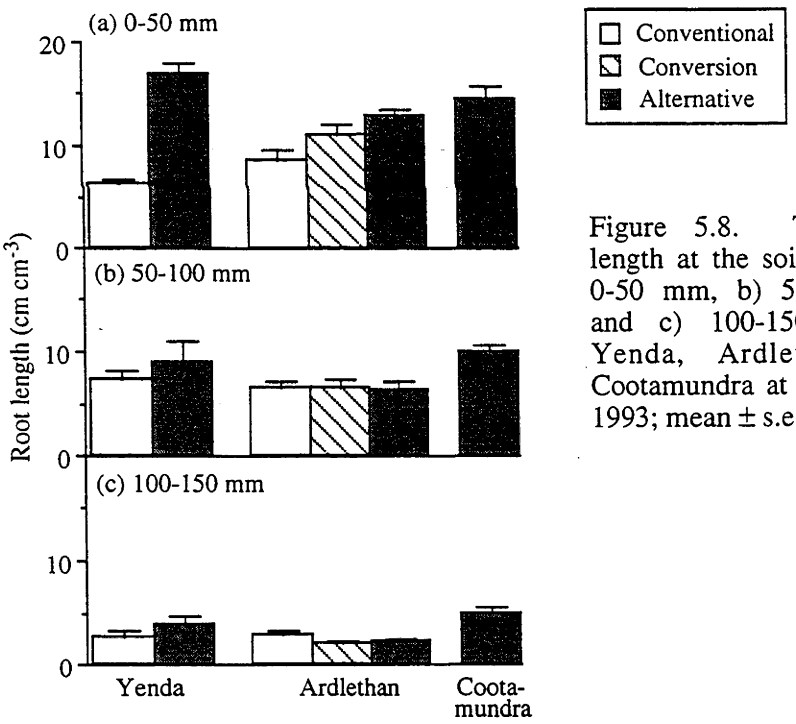


Figure 5.8. Total root length at the soil depths a) 0-50 mm, b) 50-100 mm and c) 100-150 mm at Yenda, Ardlethan and Cootamundra at anthesis in 1993; mean ± s.e.m., n=5.

Root length in the top 150 mm of soil was used to calculate the biomass of roots under each crop (Table 5.5). Root biomass was highest on the alternative farms at each location and, at Ardlethan, the conversion crop was intermediate to the organic and conventional crops. The root-shoot ratio was lowest in the two conventional crops, identical in the organic and conversion crops at Ardlethan and highest in the biodynamic crop at Cootamundra and the organic crop at Yenda (Table 5.5).

Table 5.5. Root biomass and the root-shoot ratio at anthesis in conventional (Con.), organic, conversion (Conver.) and biodynamic crops; mean *(s.e.m.)*, n=5. The root-shoot ratio was calculated using total biomass data from Derrick (1996). Both figures include crop and weeds.

	Yenda		Ardlethan			Cootamundra
	Con.	Organic	Con.	Conver.	Organic	Biodynamic
Root biomass ($t\ ha^{-1}$)	1.17 *(0.08)*	2.13 *(0.18)*	1.28 *(0.10)*	1.42 *(0.10)*	1.52 *(0.08)*	2.10 *(0.11)*
Root-shoot ratio	0.14 *(0.08)*	0.47 *(0.08)*	0.13 *(0.02)*	0.19 *(0.02)*	0.19 *(0.02)*	0.36 *(0.05)*

The concentration of P and N in the two youngest fully-emerged leaves (leaf P and N) and in the entire shoot (crop shoot P and N) at tillering are presented in Table 5.6. Leaf P was consistently higher in the conventional crops, with the conversion crop being intermediate to the conventional and alternative crops at Ardlethan; leaf N varied little between farms. Crop shoot P was generally lower than leaf P, but still tended to be higher on the conventional farms. Crop shoot N was more variable than leaf N, with no clear conventional/alternative trends.

Table 5.6. Concentration of P and N at tillering in the two youngest fully-emerged leaves (leaf P) and the entire shoot (crop shoot P) in conventional (Con.), organic, conversion (Conver.) and biodynamic crops; mean *(s.e.m.)*, n=10 (leaf), n=15 (crop). Crop shoot P from Derrick (1996).

	Yenda		Ardlethan			Cootamundra
	Con.	Organic	Con.	Conver.	Organic	Biodynamic
Leaf P	0.32 *(0.02)*	0.23 *(0.01)*	0.34 *(0.03)*	0.30 *(0.02)*	0.24 *(0.01)*	0.25 *(0.02)*
Leaf N	4.06 *(0.07)*	4.08 *(0.07)*	4.13 *(0.06)*	3.96 *(0.07)*	4.08 *(0.07)*	3.93 *(0.12)*
Crop shoot P	0.27 *(0.01)*	0.20 *(0.01)*	0.28 *(0.01)*	0.30 *(0.01)*	0.26 *(0.01)*	0.26 *(0.01)*
Crop shoot N	3.84 *(0.06)*	3.55 *(0.06)*	3.57 *(0.07)*	4.06 *(0.06)*	3.91 *(0.05)*	3.34 *(0.13)*

Due to the large differences in crop biomass, crop shoot P content followed similar trends to the crop biomass data presented in Figure 5.6. However, some additional measures of crop P uptake are presented in Figures 5.9 and 5.10.

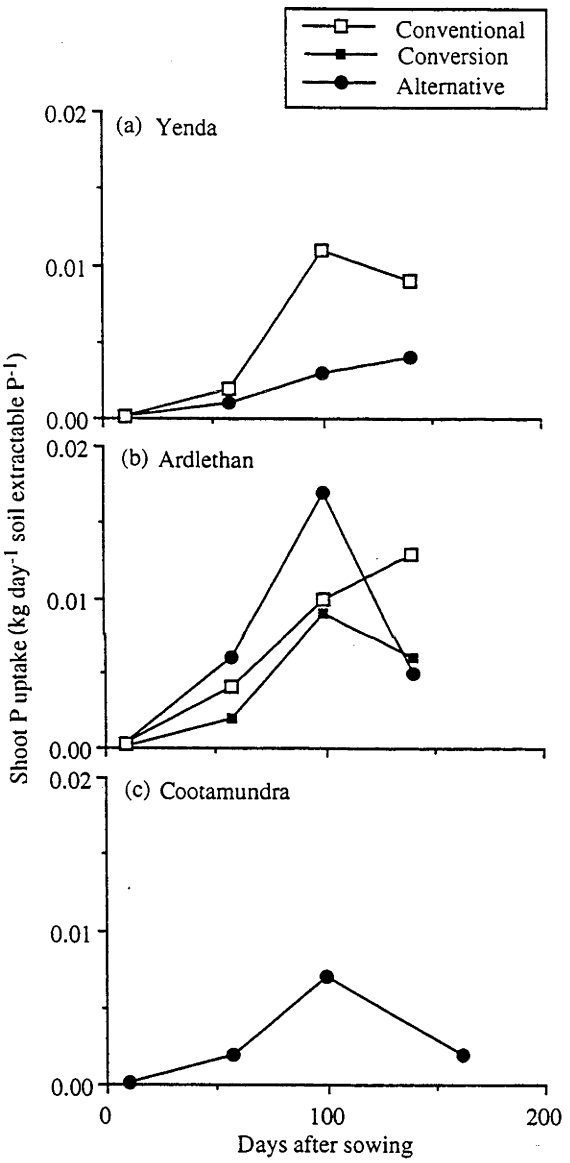


Figure 5.9. Rate of crop shoot P uptake expressed as the amount of P taken up by the wheat crops each day (kg) relative to Colwell soil extractable P ($\mu g\ g^{-1}$) over the 1993 season at a) Yenda, b) Ardlethan and c) Cootamundra. Raw data from Derrick (1996).

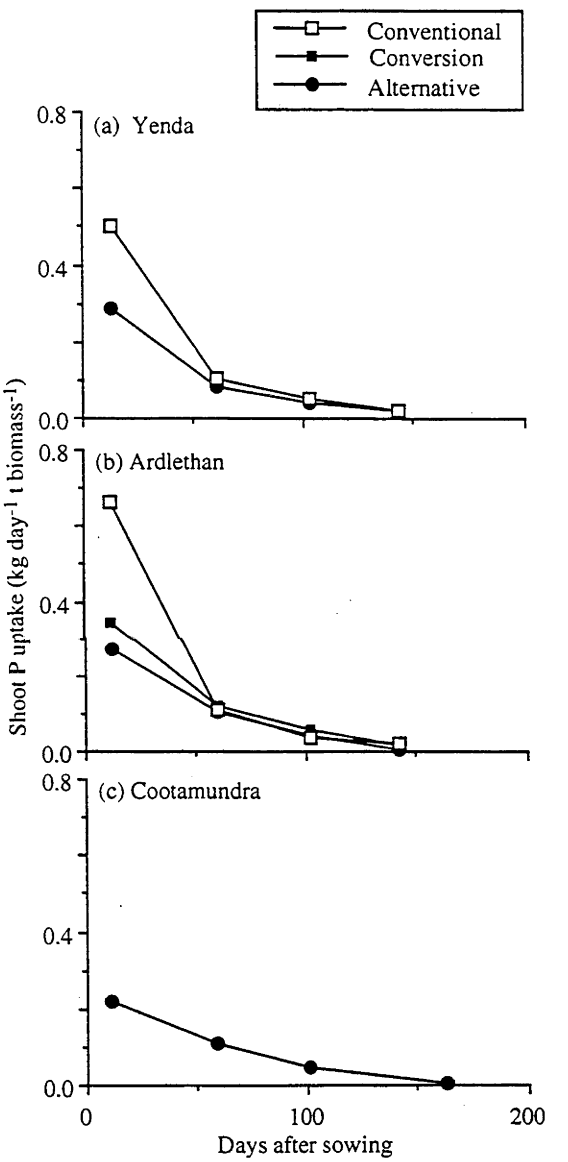


Figure 5.10. Rate of crop shoot P uptake expressed as the amount of P taken up by the wheat crops each day (kg) relative to crop shoot biomass over the 1993 season at a) Yenda, b) Ardlethan and c) Cootamundra. Raw data from Derrick (1996).

The P uptake rate relative soil extractable P (Fig. 5.9) increased over the cropping season until anthesis and then declined between anthesis and harvest on the alternative farms at Ardlethan and Cootamundra and slightly increased on the organic farm at Yenda and conventional farm at Ardlethan. The organic crop at Ardlethan — the only alternative crop not severely affected by weeds — maintained a greater rate of P uptake relative to soil available P than the conventional crop until anthesis, when the rate markedly declined.

The rate of P uptake relative to crop shoot biomass (Fig. 5.10) was highest at seedling stage, then declined markedly until tillering, before gradually decreasing over the remainder of the season. This measure of P uptake was much higher on the conventional farms than the alternative farms at the beginning of the season. While this difference was maintained until harvest at Yenda, at Ardlethan, the alternative crops had a similar or greater rate than the conventional crop mid-season, before again declining after anthesis to a much greater degree than the conventional crop.

5.3.e. Relationships between VAM Colonisation Levels, Soil and Crop Nutrient Concentrations, Crop Biomass, Weed Abundance and Farm Management Strategy

To explore the factors responsible for the results presented in sections 5.3.c and 5.3.d, the relationships between VAM (%), soil and crop nutrient concentrations, crop biomass, weed abundance and farm management strategy were examined. Three sets of statistical analyses were preformed on data collected from 15 sites in each of the six first year wheat crops: ANOVA models to examine the effect of farm location and management strategy; regression analyses to examine the effects of continuous biological or soil factors; and stepwise regressions. The stepwise regressions were designed to examine whether location and, in particular, farm management strategy, explained variation in the data in addition to that which was already explained by the continuous soil and biological variables (§3.9). Some models from the regression analyses were summarised graphically as simple regressions of paddock means. The ANOVA and regression analyses were performed separately, as the strong correlation of both farm location and farm management with biological and soil variables, such as soil extractable P, made it impossible to construct a model which included these two sets of variables and gave a meaningful indication of the amount of variation explained by each variable. Although some analysis of anthesis data occurred, most analysis occurred on data collected at tillering. As soil total P and N were not available at anthesis, values from tillering were used. Limited analysis was made of trends across the cropping season.

(i) Influence of Farm Location and Management Strategy

Data were examined using ANOVAs that included only location (Yenda, Ardlethan or Cootamundra), management strategy (conventional or alternative) and an interaction term. The conversion paddock was excluded from this analysis as it was considered to have been under organic management for insufficient time to warrant classification as alternative. Results are presented in Figure 5.11 as graphs of the interaction between location and management strategy and are also summarised in Table 5.7.

Total soil N was the only variable significantly affected by location alone and VAM colonisation was the only variable affected by management strategy alone, being consistently much higher on the alternative farms. While also affected by location, weed biomass was higher on alternative farms, and soil extractable P and total biomass higher on the conventional farms. For the remaining factors, the model contained a significant interaction between location and management strategy, that is, the effect of management strategy differed with location. The strength of this analysis is obviously limited by the small number of farms involved and the absence of a conventional farm at Cootamundra.

Table 5.7. Summary of the significant predictor variables and their level of significance when soil and biological parameters measured in five first year cropping paddocks (the conversion paddock was excluded) were fitted with location (Yenda, Ardlethan, Cootamundra), farm management strategy (conventional, alternative) and a location x management strategy interaction term. When the interaction term was significant, it alone is shown in the table. Soil and crop nutrient concentrations and biomass measures from Derrick (1996).

Factor	Location	Management strategy	Location x Management Interaction
Log soil total P	-	-	<0.0001
Soil total N	<0.0001	-	-
Log soil extractable P	<0.0001	<0.0001	-
Log VAM (%)	-	<0.0001	-
Crop shoot P	-	-	0.007
Crop shoot N	-	-	0.0001
Log crop biomass	-	-	<0.0001
Weed biomass	-	-	<0.0001
Log total biomass	<0.0001	<0.0001	-
Log root length	-	-	0.0002

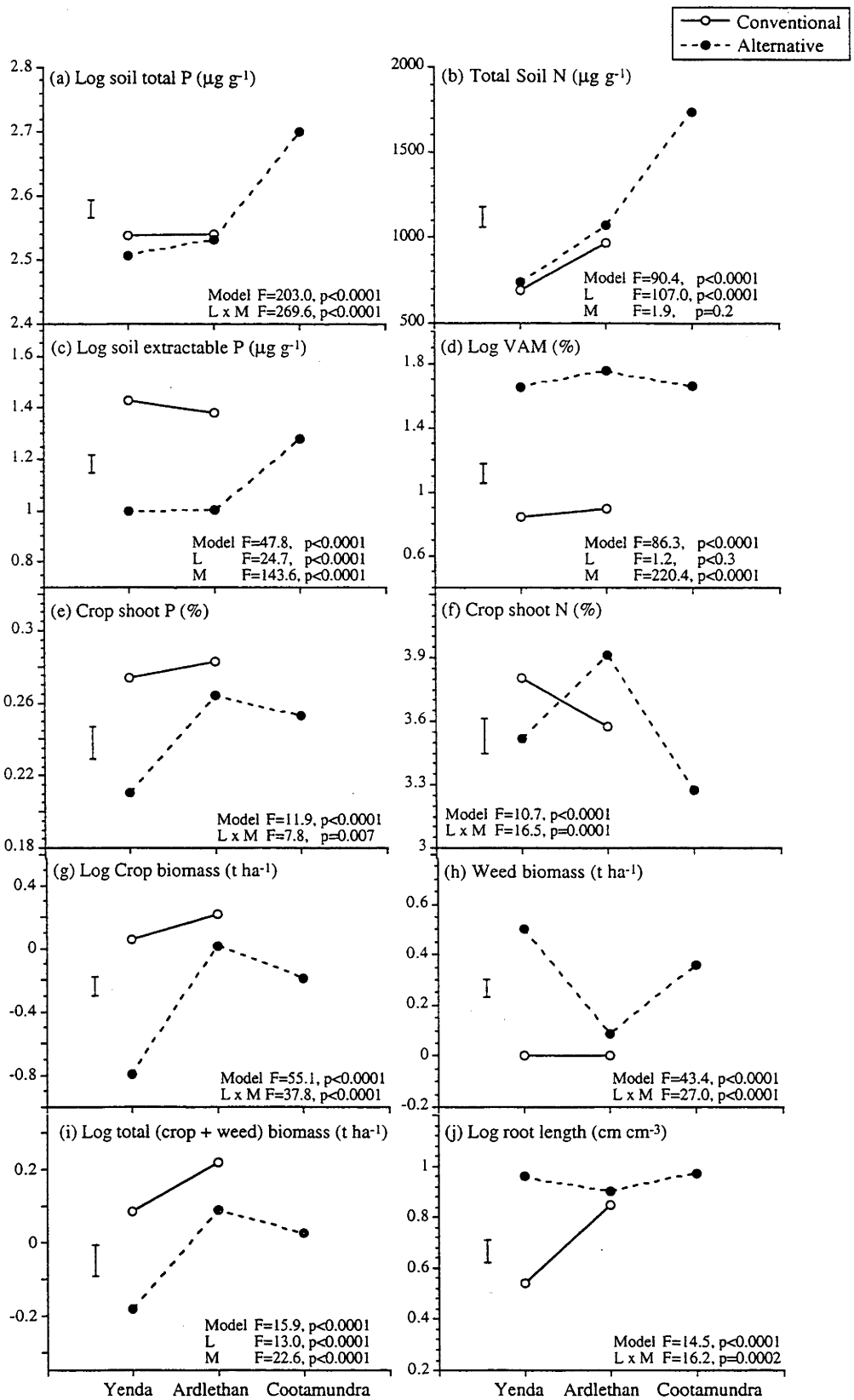


Figure 5.11. The interaction between farm location (L) (Yenda, Ardlethan, Cootamundra) and farm management (M) (conventional, alternative) for various soil and crop measures at tillering 1993 (estimated mean and LSD at p=0.05). The conversion paddock was not included. Each ANOVA model initially included Location, Management and Location x Management. When the interaction term was significant, the F-value and probability level are given for the whole model (L + M + LxM) and the interaction term only. When the interaction term was not significant it was removed and the F-value and probability levels are given for the model (L + M) and both Location and Management. All data, except (d) and (j), from Derrick (1996).

(ii) Relationships Between Continuous Soil and Biological Variables

VAM Colonisation

Table 5.8 presents the results from regression analyses of the soil and biological variables significantly influencing various measures of VAM colonisation at tillering on all six farms. The level of VAM colonisation was consistently strongly negatively correlated with P. Leaf P (Fig. 5.12.a) was a better predictor than crop shoot P, while crop shoot N had no effect on colonisation. However, VAM (%) correlated most strongly with soil variables, with soil extractable P exerting a strong negative effect (Fig. 5.12.b) and soil total N a slight positive effect.

Table 5.8. Results from statistical analysis of measures of VAM colonisation at tillering. Parameters included are Colwell soil extractable P, soil total P and soil total N ($\mu g\ g^{-1}$), colonised root length in top 50 mm of soil ($cm\ cm^{-3}$), crop shoot P (%) and leaf P (%). Soil nutrient concentrations and crop shoot P from Derrick (1996).

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
VAM (%)	full model	-	-	14.41	0.0005	0.23	45
	intercept	83.1	45.1	6.3^t	<0.0001		
	leaf P	-171.5	45.2	14.4	0.0005		
VAM (%)	full model	-	-	8.0	0.007	0.11	57
	intercept	98.4	22.3	4.41^t	<0.0001		
	crop shoot P	-240.1	84.88	8.0	0.007		
VAM (%)	full model	-	-	34.7	<0.0001	0.54	58
	intercept	53.9	8.4	6.4^t	<0.0001		
	soil extractable P	-1.97	0.26	58.7	<0.0001		
	soil total N	0.017	0.006	7.8	0.007		
Log VAM intensity	full model	-	-	38.7	<0.0001	0.53	58
	intercept	1.5	0.14	10.32^t	<0.0001		
	soil total N	0.0007	0.0001	47.7	<0.0001		
	soil extractable P	-0.03	0.005	42.1	<0.0001		
Log VAM intensity	full model	-	-	797.1	<0.0001	0.97	54
	intercept	-0.34	0.05	-6.49^t	<0.0001		
	Log VAM (%)	1.13	0.03	1133.7	<0.0001		
	Soil total N	0.0003	0.0001	62	<0.0001		
Log root length VAM	full model	-	-	34.4	<0.0001	0.56	53
	intercept	2.04	0.15	13.72^t	<0.0001		
	soil total P	-0.002	0.0001	52.7	<0.0001		
	soil total N	0.0004	0.0001	17.6	0.0001		

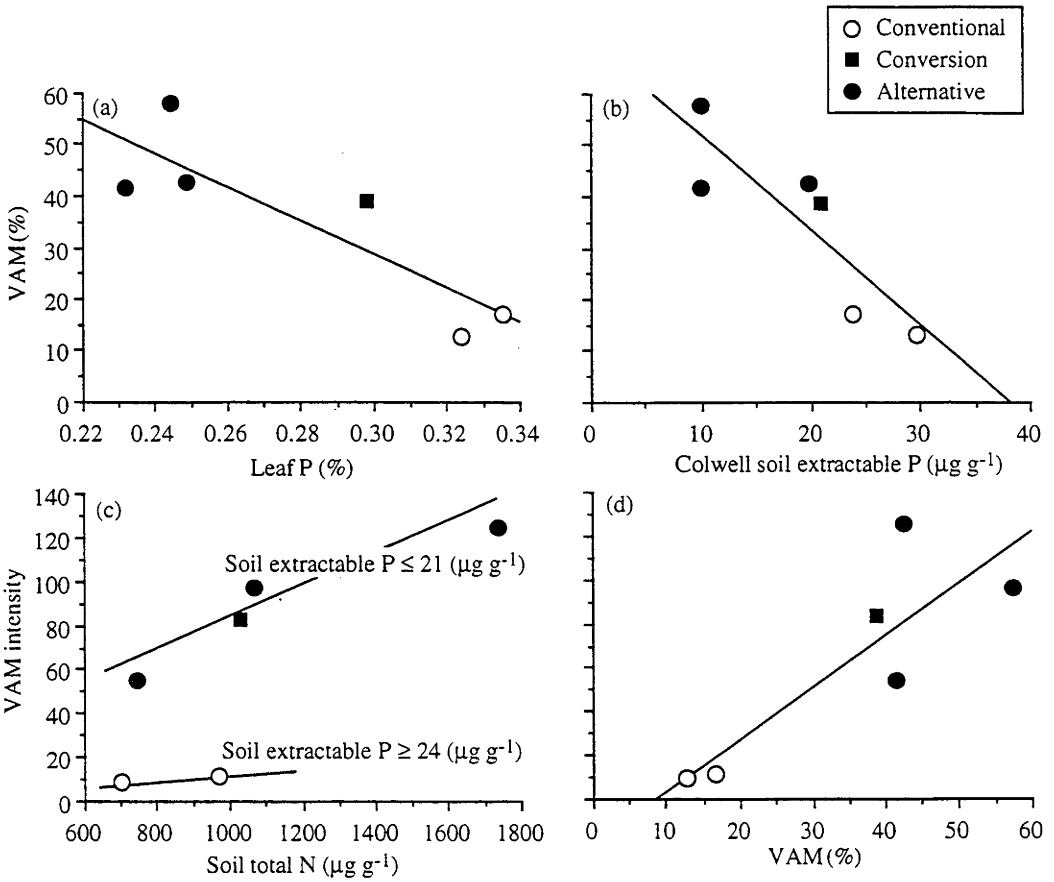


Figure 5.12. Simple regressions of the relationships at tillering between a) the percentage of root length colonised by VAM fungi and the concentration of P in the youngest two fully-emerged leaves (r^2=0.75), b) the percentage of root length colonised by VAM fungi and Colwell soil extractable P (r^2=0.74), c) VAM intensity and soil total N at low (r^2=0.91) and high levels of Colwell soil extractable P and d) VAM intensity and the percentage of root length colonised by VAM fungi (r^2=0.73). Farm management strategy (conventional, conversion, alternative) is indicated. Each point represents the average from 15 sites sampled in one of the six first year wheat crops. Soil P and N concentrations from Derrick (1996).

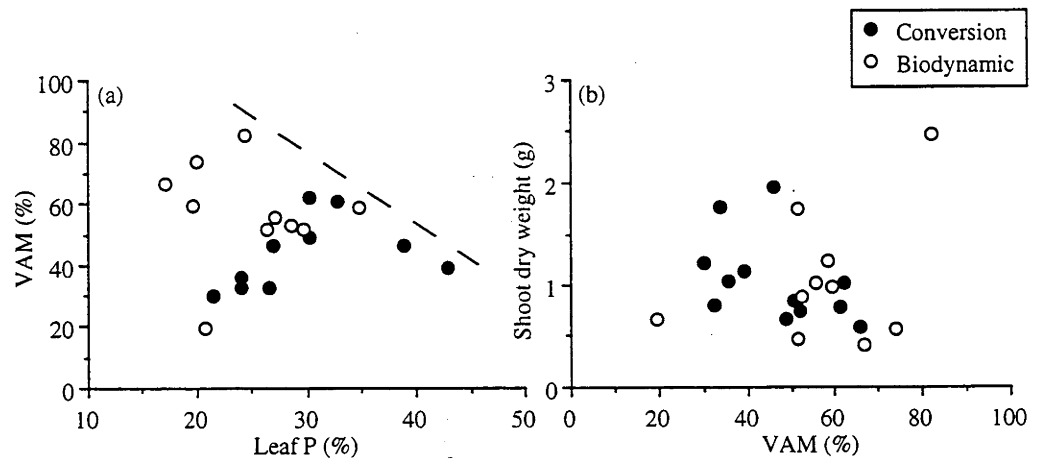


Figure 5.13. The relationship between a) the percentage of root length colonised by VAM fungi and the concentration of P in the youngest two fully-emerged leaves and b) shoot dry weight and the percentage of root length colonised by VAM fungi for 15 individual plants from the conversion and biodynamic wheat crops at tillering 1993. The dashed line indicates an apparent upper limit to VAM colonisation.

When soil variables were used to model VAM intensity, there was a strong positive effect from soil total N, as well as a negative soil extractable P effect (Table 5.8 and Fig. 5.12.c). Log VAM intensity was strongly positively correlated with VAM (%) (Fig 5.12.d), although soil total N also had a significant positive effect. The length of roots colonised by VAM fungi in the top 50 mm of soil was highly negatively correlated with soil total P and to a lesser extent positively correlated with soil total N; a reflection of the relationship between root length and total soil P and N (Table 5.10). Soil pH and rainfall did not have a significant effect in any of the models.

Leaf P and VAM (%) were measured on individual plants from the conversion crop at Ardlethan and the biodynamic crop at Cootamundra at tillering. While a broad range of VAM colonisation levels and leaf P concentrations were present, there was no significant linear relationship between these two parameters at the scale of individual plant, although there does appear to be an upper limit to VAM colonisation which increases as leaf P decreases (Table 5.9 and Fig. 5.13.a). There was no correlation between shoot dry weight and VAM colonisation (Fig 5.13.b), with shoot dry weight being affected positively by leaf P and negatively by leaf N.

Table 5.9. Results from statistical analysis of measures on individual plants from the conversion and biodynamic crops at tillering. Parameters included are percentage of root length colonised by VAM fungi, leaf P and N (%) and shoot dry weight (g).

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
VAM (%)	full model	-	-	0.07	0.8	-0.05	20
	intercept	54.5	15.8	3.4^t	0.003		
	leaf P	-15.4	56.4	0.07	0.8		
Log shoot weight	full model	-	-	7.8	0.004	0.43	19
	intercept	0.98	0.5	2.0^t	0.06		
	leaf P	2.29	0.7	10.5	0.005		
	leaf N	-0.41	0.1	9.8	0.006		

Figure 5.14 presents the relationship between VAM (%) and both shoot P and soil extractable P, at seedling, tillering and anthesis. Both shoot P and soil extractable P appear to strongly impose an upper limit on colonisation, severely limiting colonisation when high and allowing a range of colonisation levels when low.

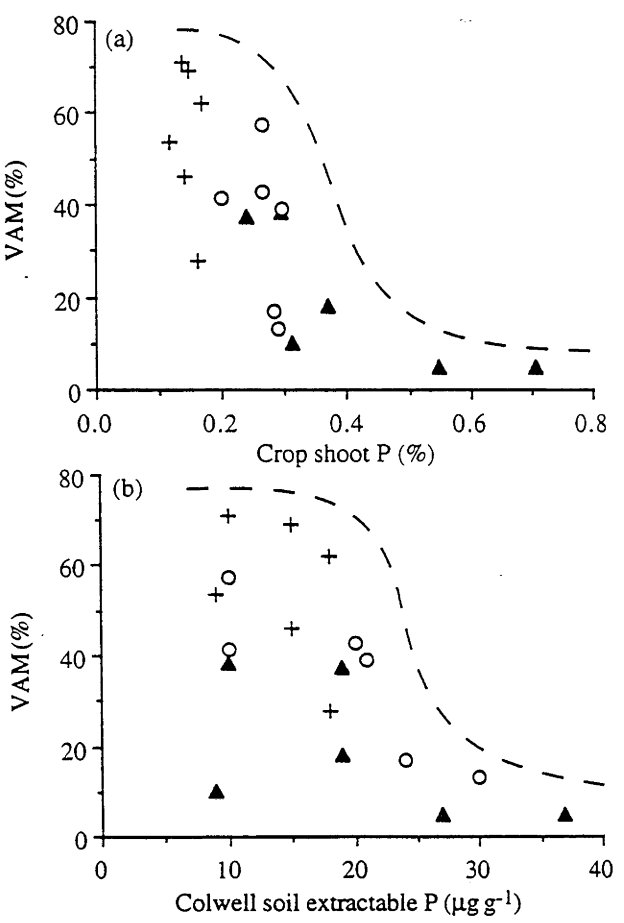


Figure 5.14. Relationship between percentage of root length colonised by VAM fungi at seedling, tillering and anthesis and a) crop shoot P concentration and b) Colwell soil extractable P. The dashed line indicates an apparent upper limit to VAM colonisation. Each point represents the average from 15 sites sampled in one of six first year wheat crops in 1993. Soil and crop shoot P concentrations from Derrick (1996).

Crop Growth

Table 5.10 presents regression models of various indicators of crop growth at tillering. Crop shoot biomass was best predicted using crop shoot nutrient concentrations and weed biomass. Weed biomass exerted a strong negative influence (Fig. 5.15), while crop shoot P had a positive effect and crop shoot N a negative effect. When soil nutrients replaced crop shoot nutrients in the model, weed biomass was still the major determinant of crop biomass, with soil extractable P exerting a slight positive effect. Soil total N was positively, but not significantly, correlated with crop biomass in this model. Root length in the top 50 mm of soil was strongly negatively correlated with total soil P, while total soil N had a slight positive effect; soil extractable P did not exert a significant effect. Crop shoot P was positively correlated with soil extractable P and the crop P content was positively affected by soil extractable P and negatively affected by weed biomass. Rainfall, VAM (%), VAM intensity and the length of root colonised by VAM fungi did not significantly effect any of the dependent variables.

Table 5.10. Results from statistical analysis of measures crop growth at tillering. Parameters included are crop and weed biomass (t ha^{-1}), Colwell soil extractable P, soil total P and total N (μg g^{-1}), colonised root length in top 50 mm of soil (cm cm^{-3}), crop shoot P and N (%) and crop shoot P content (kg ha^{-1}). All data, except root length, from Derrick (1996). VAM colonisation and rainfall did not exert an influence in any of the models.

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
Crop biomass	full model			42.7	<0.0001	0.60	86
	intercept	1.60	0.46	3.48[t]	0.0008		
	weed biomass	-1.69	0.21	64.1	<0.0001		
	crop shoot P	7.6	1.2	39.7	<0.0001		
	crop shoot N	-0.62	0.14	20.4	<0.0001		
Crop biomass	full model	-	-	43.7	<0.0001	0.50	87
	intercept	0.90	0.13	7.17[t]	<0.0001		
	weed biomass	-1.56	0.21	53.8	<0.0001		
	soil extractable P	0.02	0.005	16.6	0.0001		
Log root length	full model	-	-	29.8	<0.0001	0.52	55
	intercept	3.00	0.08	38.7[t]	<0.0001		
	soil total P	-0.0008	0.00012	44.7	<0.0001		
	soil total N	0.0002	0.00005	14.9	<0.0001		
Crop shoot P	full model	-	-	50.52	<0.0001	0.37	86
	intercept	0.22	0.008	26.8[t]	<0.0001		
	soil extractable P	0.003	0.0004	50.52	<0.0001		
Crop total P content	full model	-	-	51.3	<0.0001	0.53	86
	intercept	0.18	0.03	5.18[t]	<0.0001		
	weed biomass	-0.39	0.06	41.2	<0.0001		
	soil extractable P	0.008	0.001	39.3	<0.0001		

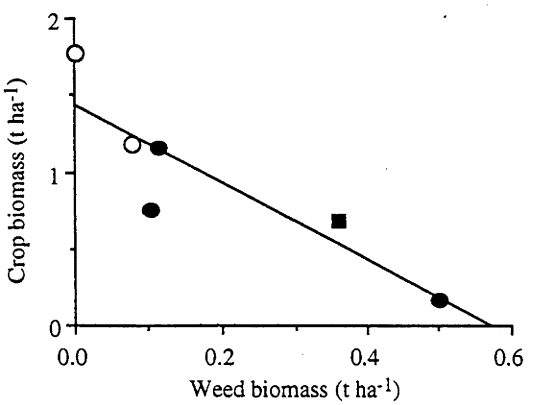


Figure 5.15. Simple regression of the relationship at tillering between crop biomass and weed biomass. Farm management strategy (conventional, conversion, alternative) is indicated. Each point represents the average from 15 sites sampled in one of the six first year wheat crops in 1993. Data from Derrick (1996).

A limited examination was made of data collected at anthesis (Table 5.11). The percentage of root length colonised by VAM fungi was influenced only by soil extractable P, however the model did not explain as much variation in the data as it did at tillering. Soil total N and crop shoot nutrient concentrations no longer had a significant effect on VAM (%). VAM intensity did not correlate significantly with any factors. Crop biomass was again best described by a strong negative weed biomass effect and a positive soil extractable P effect. The root-shoot ratio — where measures of both roots and shoots included crop and weeds — was strongly negatively correlated with log total biomass and positively correlated with log root biomass (Fig. 5.16). The root-shoot ratio was also positively correlated with weed biomass, but was not significantly influenced by soil nutrient concentrations.

Table 5.11. Results from statistical analysis of the percentage of root length colonised by VAM fungi, crop biomass and root-shoot ratio at anthesis. Parameters included are crop, weed and total biomass (t ha^{-1}), Colwell soil extractable P (μg g^{-1}) and root biomass (t ha^{-1}). All data, except VAM colonisation and root biomass, from Derrick (1996).

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
VAM (%)	full model	-	-	31.0	<0.0001	0.26	86
	intercept	82.6	5.4	15.3^t	<0.0001		
	soil extractable P	-2.17	0.39	31.0	<0.0001		
Crop biomass	full model	-	-	54.1	<0.0001	0.70	86
	intercept	4.95	0.63	7.85^t	<0.0001		
	weed biomass	-0.41	0.13	109.5	<0.0001		
	soil extractable P	0.24	0.04	38.9	<0.0001		
Root-shoot ratio	full model	-	-	71.0	<0.0001	0.71	29
	intercept	1.02	0.09	10.9^t	<0.0001		
	log_{10} total biomass	-0.88	0.1	71.0	<0.0001		
Root-shoot ratio	full model	-	-	43.3	<0.0001	0.60	29
	intercept	0.27	0.02	14.6^t	<0.0001		
	log_{10} root biomass	1.01	0.15	43.3	<0.0001		
Root-shoot ratio	full model	-	-	15.2	0.0005	0.33	30
	intercept	-0.822	0.051	-16.01^t	0.001		
	weed biomass	0.097	0.025	15.2	0.0005		

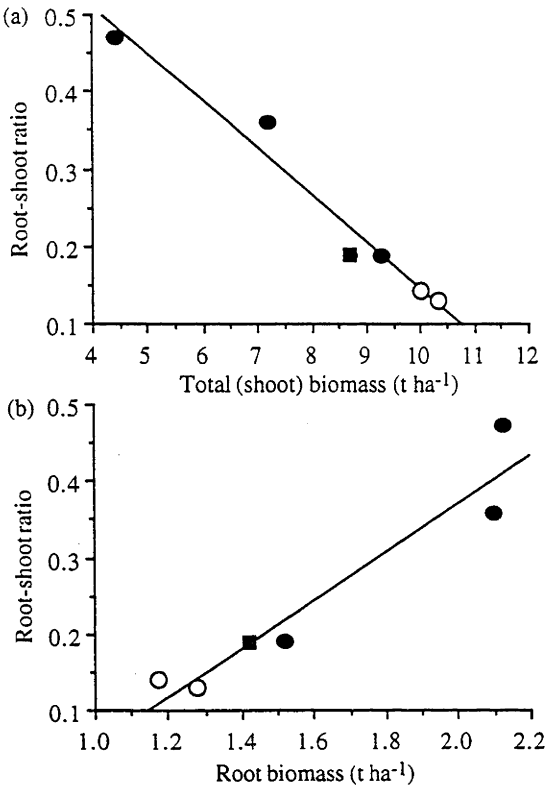


Figure 5.16. Simple regression of the relationship at anthesis between the root-shoot ratio and a) total shoot biomass (r^2=0.96) and b) root biomass (r^2=0.92). All measures included both crop and weeds. Farm management strategy (conventional, conversion, alternative) is indicated. Each point represents the average from five sites sampled in one of the six first year wheat crops in 1993. Shoot biomass from Derrick (1996).

(iii) Comparison of Relationships Between Farm Management Strategies

To investigate whether the relationships between soil nutrients, crop biomass and VAM (%) at tillering were similar on farms under different management strategies, a stepwise regression was performed. The continuous variables used in the models in Tables 5.8 and 5.10 were added first, followed by location and management strategy.

In the first analysis, only data from the four alternative farms was used, with management strategy being divided into organic, conversion and biodynamic (Table 5.12). Neither location or management strategy had a large influence on the fit of the model for VAM (%), while location did markedly increase the adjusted r^2 for crop biomass, with management strategy exerting a smaller effect. As the relationships between variables appeared to be similar under the three alternative management strategies, in the second analysis they were all classified as alternative. Thus in the second analysis (Table 5.13), data from all six farms were used. For VAM (%), addition of management strategy resulted in a substantial increase in the adjusted r^2, while location had no effect. For crop biomass, the addition of both location and management strategy to the model increased r^2 by only a negligible amount.

Table 5.12. Results from a stepwise regression analysis designed to investigate whether location (Yenda, Ardlethan, or Cootamundra) or the type of alternative management (organic (O), conversion (c) or biodynamic (BD)) was affecting the percentage of root length colonised by VAM fungi or crop biomass in a manner other than through their influence on weed biomass, Colwell soil extractable P and soil total N. Data from the four alternatively managed first year wheat crops at tillering were used. The continuous variables were added first in order of the size of their influence, and location and farm management were then added and the cumulative adjusted r^2, and the change in r^2, both recorded.

Dependent variable	Predictor variables	Adjusted r^2	Change in r^2
VAM (%)	soil extractable P	0.16	+16
	+ soil total N	0.18	+2
	+ *location*	*0.20*	*+2*
	+ *management strategy (O/c/BD)*	*0.17*	*-3*
Log crop biomass	weed biomass	0.35	+35
	+ soil extractable P	0.55	+20
	+ soil total N	0.57	+2
	+ *location*	*0.68*	*+11*
	+ *management strategy (O/c/BD)*	*0.72*	*+4*

Table 5.13. Results from a stepwise regression analysis designed to investigate whether farm location (Yenda, Ardlethan, or Cootamundra) or farm management strategy (conventional (C), or alternative (A) with alternative including the conversion, biodynamic and two organic crops) were affecting the percentage of root length colonised by VAM fungi or crop biomass in a manner other than through their influence on weed biomass, Colwell soil extractable P and soil total N. Data from all six first year wheat crops at tillering were used. The continuous variables found to be significant in the analyses in Tables 5.8 and 5.10 were added first in order of the size of their influence, and location and farm management were then added and the cumulative adjusted r^2, and the change in r^2, both recorded.

Dependent variable	Predictor variables	Adjusted r^2	Change in r^2
VAM (%)	soil extractable P	0.49	+49
	+ soil total N	0.54	+ 5
	+ *location*	*0.53*	*- 1*
	+ *management strategy (C/A)*	*0.68*	*+15*
Log crop biomass	weed biomass	0.50	+50
	+ soil extractable P	0.59	+ 9
	+ soil total N	0.63	+ 4
	+ *location*	*0.68*	*+ 5*
	+ *management strategy (C/A)*	*0.70*	*+ 2*

5.4. Discussion

5.4.a. Soil Nutrients and Rainfall

Soil extractable P was higher on the conventional farms, presumably reflecting the addition of fertilisers containing soluble P. However, the biodynamic farm at Cootamundra also had quite high extractable P which was probably due to underlying soil characteristics, as in 1995 a conventional neighbour was also sampled and found to have high extractable P (40 $\mu g\ g^{-1}$). Total soil N varied primarily with location, increasing both as farms were located further east and as rainfall increased. At Ardlethan, the higher pH on the organic farm was due to past applications of lime.

In 1993, annual and growing season rainfall was above average at all three locations and it is unlikely that lack of water was limiting yield on any of the farms (Cornish and Murray 1989). Rainfall in the four months preceding sampling was not found to be a significant factor in the models of crop biomass at tillering and anthesis (Tables 5.10 and 5.11).

5.4.b. Factors Influencing VAM Colonisation Levels

(i) Farm Management Strategy and Farm Location

VAM colonisation was the only parameter solely affected by management strategy irrespective of location (Table 5.7), with VAM (%) in the alternative crops always being higher than on conventional neighbours (Fig. 5.1). This finding reinforces the preliminary work of Ryan (1992) at Ardlethan and the work of researchers in other countries (Bokhorst 1989; Sattelmacher *et al.* 1991; §12.3.a).

(ii) Soil Factors, Biological Factors and Farm Management Practices

At tillering and anthesis, VAM colonisation levels were most strongly affected by P, with increasing P corresponding with decreasing colonisation. This is the standard response of VAM colonisation to increasing P (Smith and Read 1997). At tillering (Table 5.8), VAM colonisation correlated most strongly with soil parameters, with extractable P strongly decreasing colonisation and total N having a slight positive effect. Soil total N had a much larger positive effect on VAM intensity; perhaps low N was limiting the formation of arbuscules to some degree, while high P was inhibiting the spread of colonisation through the root system. Nitrogen deficiency has been found to decrease VAM colonisation by Vejsadová *et al.* (1989), although in 1993, N concentrations were adequate in all crops (§5.4.e.ii).

VAM colonisation also correlated negatively with the concentration of P in the crops, with the concentration in the youngest two fully-emerged leaves (leaf P) being a better predictor than the concentration in the entire shoot (crop shoot P). VAM colonisation is known to be controlled by the concentration of P in the roots (Bruce

et al. 1994; Lu *et al.* 1994; see also Fig. 10.7.c). Presumably, in an actively growing plant, leaf P would better reflect root P than the average P concentration in the entire shoot (see §12.1.a.ii). The correlation between P and VAM was not present at the level of individual plants (Table 5.9 and Fig. 5.13). Presumably, factors such as leaf age, plant health and VAM inoculum levels were obscuring the relationship at this finer scale. This issue is discussed further in section 12.1.a.x.

A negative effect from soluble P fertilisers on VAM colonisation is evident in Figure 5.4 where VAM colonisation on the conventional farms was greater in the inter-rows than on the rows which contained banded soluble P fertiliser. Lu *et al.* (1994) found that banding fertiliser decreased colonisation in roots growing in fertiliser, but had less effect on roots outside the fertiliser band.

Overall, the lower VAM colonisation levels on the conventional farms would seem to reflect the higher soil extractable P on these farms, due to the use of fertilisers containing soluble P, such as superphosphate. This was also indicated by the results of Dann *et al.* (1996) (Fig. 5.17). In fertiliser trials on the conventional (I) and organic farms at Ardlethan in 1992, Dann *et al.* (1996) found the addition of rock phosphate did not result in VAM (%) differing significantly from the unfertilised control, while the addition of superphosphate resulted in a large reduction in VAM (%). In the control treatments, the lower VAM colonisation on the conventional farm seems to indicate that factors other than fertiliser addition were reducing colonisation on the conventional farm.

In the stepwise regression of data from all six farms (Table 5.13), soil extractable P explained 40% of the variation in VAM colonisation at tillering and soil total N an additional 5%, however, management strategy explained an additional 14% of variation. When the alternative farms were examined alone, addition of management strategy to the model did not explain any additional variation (Table 5.12). This also appears to indicate that while conventional farm management was decreasing VAM colonisation at tillering primarily due to addition of soluble P fertilisers and an underlying higher concentration of extractable soil P, other factors were also involved.

Tillage may reduce VAM colonisation through disrupting the hyphal network (Evans and Miller 1988; Evans and Miller 1990; Jasper *et al.* 1989), however tillage types and frequencies were similar on the conventional and alternative farms (Table 5.1). Other factors with the potential to reduce VAM colonisation levels on conventional farms were examined by Ryan (1992) in a glasshouse trial using soil from the organic farm at Ardlethan (Fig. 5.18). Dressing seeds with fungicide, herbicide application, N fertiliser and wheat variety all had no significant effect on the VAM (%); although N did slightly increase the colonisation level. It is possible that the effects of these could differ under field conditions. Alternatively, the wheat on the conventional farms may have been less colonised than predicted from the soil extractable P

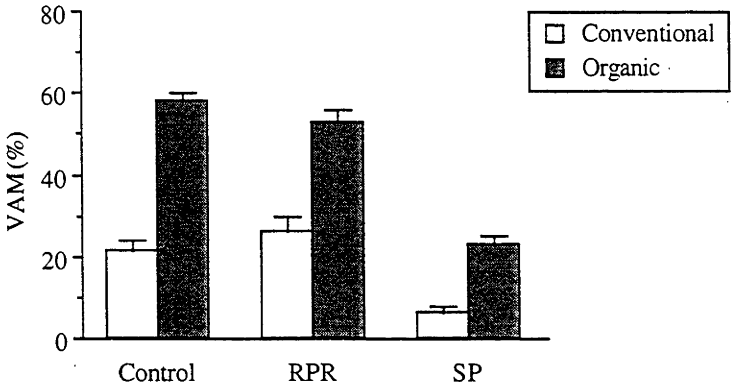


Figure 5.17. Percentage of wheat root length colonised by VAM fungi on fertiliser trials on the conventional I and organic farms at Ardlethan at tillering 1992. Treatments were a control (no fertiliser) and 40 kg ha^{-1} of P as reactive phosphate rock (RPR) and superphosphate (SP); mean ± s.e.m., n=20. Adapted from Dann *et al.* (1996).

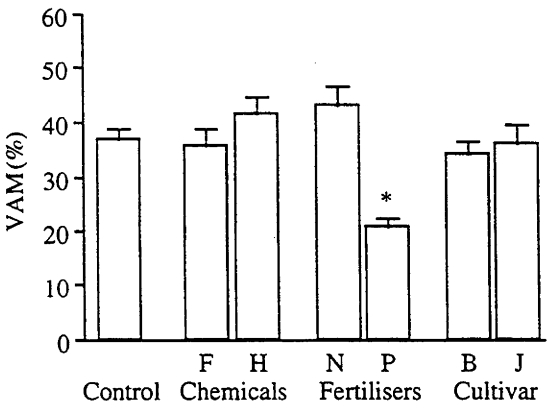


Figure 5.18. Percentage of wheat root length colonised by VAM fungi in a glasshouse trial using soil from the organic farm at Ardlethan; mean ± s.e.m., n=15. Treatments using wheat cv. Vulcan were the control, fungicide seed dressing Vitavax® (F), herbicides Hoegrass® and Jaguar® (H), 0.111 g pot^{-1} of NH_4NO_3 (N) and 0.0447 g pot^{-1} of NaH_2PO_4 (P). Wheat cv. Banks (B) and cv. Janz (J) were also included. * indicates that the treatment was significantly different from the control at $p<0.05$. Adapted from Ryan (1992).

concentrations due to the plants at tillering still maintaining most of their roots close to the crop rows — and obtaining most of their P from the banded fertiliser under the crop rows — while the measure of soil extractable P was an average from the rows and inter-rows. Fertiliser was not banded, and relatively insoluble, on the alternative farms.

It is also possible that colonisation was reduced on the conventional farms in comparison to the alternative farms due to lower levels of VAM inoculum in the soil or perhaps a different species of VAM fungi dominating; a species with a slower colonisation rate and a lower maximum colonisation level (see §12.3.c.i).

(iii) Dynamics of VAM Colonisation over the Cropping Season

The percentage of root length colonised by VAM fungi, and VAM intensity, peaked in all crops at anthesis, with VAM intensity then declining at Ardlethan and Cootamundra. Although no quantitative measures were made, it was noted that at seedling and tillering colonisation primarily consisted of arbuscules. However by anthesis, there were large sections of root containing only vesicles and hyphae, and by harvest, few arbuscules remained. Also, while there was a strong negative relationship between P and VAM (%) in seedlings, by anthesis this was not evident. Soil extractable P and shoot P were low in all crops by anthesis, yet VAM colonisation on the conventional farms had not increased to the levels present on the alternative farms (Fig. 5.14).

This may indicate that by anthesis both the growth and activity of the VAM fungi had declined, perhaps due to a decrease in the carbohydrates supplied from the host plants. Root biomass is likely to peak around anthesis (Pearson *et al.* 1991) and the majority of plant P uptake is likely to occur before the end of tillering (Römer and Schilling 1986). Thus, while colonisation levels may have been limited by high P concentrations at the beginning of the cropping season, by anthesis other factors, including host plant physiology, may have started to restrict VAM growth and triggered the replacement of arbuscules with vesicles.

5.4.c The Role of VAM Fungi in Crop Growth

All measures of VAM colonisation differed most between the conventional and alternative crops at tillering. Phosphorus uptake by wheat is greatest until the end of tillering and the majority of P required for maximum yield is absorbed during these early growth stages (Römer and Schilling 1986; Sutton *et al.* 1983). Thus if VAM fungi were to significantly influence the P nutrition of the wheat crops, colonisation would need to quickly reach a high level and remain there, at least until tillering. This was the case only on the alternative farms.

Indeed the greater percentage of root length colonised, and the far greater length of colonised roots under the alternative crops suggests that if VAM fungi were influencing crop growth, they were likely to have played a much larger role on the

alternative farms. Figures 5.9 and 5.10 possibly indicate different P uptake dynamics on the alternative and conventional farms. Of most interest are the conventional and organic crops at Ardlethan; the only paired comparison where the organic crop was not significantly affected by weeds. While P uptake relative to crop biomass was initially far greater in the conventional crop, presumably due to easily accessible soluble P fertiliser, it was then fairly similar for the two crops for the rest of the season. However, P uptake relative to soil extractable P was far greater in the organic crop until after anthesis, when it dropped markedly. This may reflect the influence of VAM fungi, as VAM activity also appeared to drop markedly after anthesis (§5.4.b.iii).

However, it is possible that other factors — such as differences in wheat variety, the physiological response of wheat to decreasing soil P concentrations or the greater root biomass on the alternative farms — were responsible for these results (see Baon *et al.* 1993a). In addition, the quadrat size used to assess crop biomass by Derrick was small and may have lead to inaccuracies in estimating biomass (Derrick 1996). While the large differences in crop biomass, relative to the s.e.m.s, shown in Figure 5.6, suggest that any error from quadrat size is likely to be small relative to the differences between crops, it may decrease the accuracy of the P uptake trends shown in Figures 5.9 and 5.10.

There was no evidence from the models presented in section 5.3.d that VAM fungi were contributing to crop growth (see §12.3.d.v). However, the lack of non-VAM controls and the simultaneously occurring positive relationship between crop growth and P and negative relationship between P and VAM (%) make it impossible to calculate the contribution of VAM fungi to crop growth. The glasshouse trial presented in Chapter 7 was designed to further explore this question.

5.4.d. Alternative Methods of VAM Assessment

VAM intensity at tillering was strongly correlated with VAM (%) (Table 5.8), although soil total N was found to have a much stronger positive influence on VAM intensity. However, due to the tendency of the alternative crops to have higher VAM intensity (Fig. 5.3), VAM intensity magnified the differences evident in VAM (%) between conventional and alternative crops (Fig. 5.2). The decrease in VAM (%) in some crops at the end of the cropping season was also magnified, presumably due to intense arbuscular colonisation being reduced to less intense colonisation consisting of hyphae and vesicles. Arbuscules have a development cycle, development through to degeneration, of only seven days (Alexander *et al.* 1988), thus presumably colonisation intensity can decline very rapidly.

Expressing VAM colonisation as root length colonised also magnified the differences observed in the VAM (%) results, as the alternative crops had both a higher VAM (%) and greater root length than the conventional crops (Fig. 5.5).

Thus both VAM intensity and length of colonised root indicated a larger difference in VAM activity between the conventional and alternative crops than was suggested by VAM (%). However, the trends shown by the two alternative measures of VAM colonisation were very similar to those shown by VAM (%) and none of the three measures correlated with crop growth (see §12.1.a.i for further discussion).

5.4.e. Factors Influencing Crop Growth

(i) Farm Management Strategy and Farm Location

Although there was a significant interaction between farm management strategy and location, crop biomass and crop P concentrations were higher on the conventional farms, while weed biomass was lower on the conventional farms (Fig. 5.11). Total biomass was higher on the conventional farms, while root length tended to be higher on the alternative farms. Crop shoot N showed no clear trend. The lack of a conventional neighbour at Cootamundra and the small number of farms sampled do not allow strong conclusions to be made about the differences between conventional and alternative farms or the effects of location. However, it appears that use of herbicides to control weeds and application of fertilisers containing soluble P were resulting in greater crop growth and yield on the conventional farms. This is discussed further in the next section.

(ii) Soil Factors, Biological Factors and Farm Management Practices

Crop Shoot Biomass

At both tillering and anthesis, weeds were the factor most strongly influencing crop growth (Tables 5.10 and 5.11). The influence of competition by weeds on crop growth and yield in Australia has been well documented. For instance, Kohn *et al.* (1966) found that for each increase of 100 g m^{-1} in weed yield there was a corresponding decrease of approximately 36 g m^{-1} in grain yield. In field trials assessing the effect of annual rye grass (*Lolium rigidum* Gaud.) on wheat yield, Reeves (1976) found that significant reductions in crop dry weight occurred within three weeks of sowing.

Both crop shoot P and soil extractable P, which were strongly correlated, were positively correlated with crop biomass. Normal leaf P is 0.30-0.45% (Reuter and Robinson 1986). Thus, the two conventional crops had adequate P, while P was likely to have been limiting growth of the biodynamic crop at Cootamundra (0.25%), both organic crops (0.23% and 0.24%) and possibly the conversion crop at Ardlethan (0.30%; Table 5.6). These results reflect the use of fertilisers containing soluble P on the conventional crops while the alternative crops received P fertilisers of relatively less solubility, such as rock phosphate. The importance of P availability for wheat growth at Ardlethan was shown by Dann *et al.* (1996) in fertiliser trials where the application of superphosphate significantly increased growth and yield of wheat in two successive

seasons, while rock phosphate application had no effect on crop growth.

Soil total N was not found to be affecting crop growth, although total N may not give an accurate indication of the N available to crops. However, as normal leaf N is 3.6-5.5% (Reuter and Robinson 1986), N appears unlikely to have been limiting growth of any of the crops, as concentrations ranged from 3.9-4.1%. Dann *et al.* (1996) also found that N did not limit growth of first year wheat at Ardlethan. All six crops sampled in 1993 were in paddocks which had been under pasture containing subterranean clover (*Trifolium subterraneum* L.) for at least three years; this should have provided adequate N for at least one crop. The use of subterranean clover in 5-8 year pasture phases should provide sufficient soil N to support up to three subsequent cereal crops (A. Ellington quoted in Coventry *et al.* 1985). The negative correlation between crop growth and crop shoot N at tillering (Table 5.11 and Fig. 5.9) may have reflected accumulation of N in the crops due to P limiting crop growth.

The stepwise regression in Table 5.13 indicated that about 70% of the variation in the crop biomass data was explained by weeds along with soil extractable P and total N, while significant amounts of remaining variation could not be explained by factors that varied consistently with location or management strategy. Factors which may affect growth of wheat on the farms at Yenda and Ardlethan are comprehensively reviewed by Derrick (1996). Variation in other factors such as wheat variety, seed rates, seed treatment, fertiliser placement were not considered to have significantly influenced the differences in crop growth between the conventional and alternative farms in 1993 (Derrick 1996).

There are few studies which compare yields between organic and conventional farms in Australia. Two studies report results from cereal cropping systems. Derrick (1996) studied the farms at Ardlethan from 1991-1993 and found yields to be consistently higher on the conventional farm. Wynen (1994b) examined 20 pairs of farms and reported higher yields on 50% of conventional farms. However, Wynen (1994b) does not state how yields were calculated and presumably they represent farmer estimates. In addition, no information is given on the location of the farms or on whether paddocks were matched by rotation stage. This project and the results of Derrick (1996) and Dann *et al.* (1996) suggest that for Australian cereal cropping systems on soils where P is limiting crop growth, conventional farms should consistently produce greater yields than alternative farms.

Root Biomass and Root-Shoot Ratio

By anthesis, when root length should be at its maximum (Pearson *et al.* 1991; Rickert *et al.* 1987), the root length in the top 50 mm of soil ranged from 6.3-17.0 cm cm^{-3} (Fig. 5.9); values consistent with the results of Pearson (1991) who sampled wheat on a red brown earth at Forbes, NSW. The highest root lengths were on the organic farm at

Yenda with 17.0 cm cm^{-3} and the biodynamic farm at Cootamundra with 14.5 cm cm^{-3}. While it is likely that the roots were competing for mobile ions at this density, uptake of relatively insoluble ions, such as P, would still have been facilitated (Newman and Andrews 1973) (further discussion is presented in §12.1.b.ii).

Root-shoot ratios were greatest on the alternative farms. There was a strong negative relationship between the root-shoot ratio and total above-ground biomass and a strong positive relationship between the root-shoot ratio and root biomass at anthesis (Fig. 5.16). Thus the proportion of biomass being allocated to root growth decreased as shoot biomass increased and increased as root biomass, and weed biomass, increased. Even though P was limiting the growth of some crops, there was no evidence from the statistical analyses of a negative relationship between soil extractable P and the root-shoot ratio (see Riley *et al.* 1993). As a general rule, the root-shoot ratio of herbaceous plants decreases with plant age and/or size (Wilson 1988b). Thus it is possible that on the alternative farms, the combination of low P and high weeds resulted in a skewing of the root-shoot ratio in favour of roots. On the conventional farms, where P was not limiting crop growth, the larger size of the plants may have resulted in a skewing of the root-shoot ratio towards shoots.

5.4.f. Were the Conventional and Alternative Farms Functioning through Similar Biological Relationships?

This question was addressed through the stepwise regressions presented in Tables 5.12 and 5.13. Around 70% of variation in crop growth was explained by measures commonly used in conventional agricultural science and the remaining variation was not explained by factors which varied consistently between conventional and alternative farms. However, the addition of management strategy did improve the adjusted r^2 for VAM colonisation from 0.54 to 0.68; the reasons for this were discussed in section 5.4.b.ii (see also §12.3.c.i). When the alternative farms were divided into organic, conversion and biodynamic, addition of management strategy did not explain any significant additional amount of variation for either crop biomass or VAM colonisation.

Thus there was little indication that the alternative farms were operating through the actions of biological processes differing from those present on the conventional farms, or that the conversion paddock was experiencing a yield reduction due to biological processes not having adjusted to alternative management (see §2.1.a, §2.3.c and §12.3.c). Crop biomass and yield of the conversion crop at Ardlethan were lower than for the neighbouring organic crop, due to a large biomass of weeds. If the crop had been under conventional management, the weeds could have been virtually eliminated through use of herbicides. The neighbouring organic crop had low weed levels, however, the potential for high levels of weeds is not necessarily reduced by long term organic management as high weed levels were present in the organic wheat at Yenda and in the organic wheat crop at Ardlethan in 1995 (§8.3).

5.5. Conclusions about First Year Cereal Crops on Mixed Farms

• The level of VAM colonisation was negatively correlated with soil extractable P and crop shoot P, although this relationship weakened as the season progressed. Soil total N had a relatively small positive effect.

• VAM colonisation was consistently higher in the alternatively managed crops, due to the use of fertilisers containing soluble P on the conventional farms.

• The total length of root colonised by VAM fungi and the intensity of VAM colonisation were both strongly correlated with the percentage of root length colonised by VAM fungi, although VAM intensity exhibited a much stronger positive correlation with soil total N.

• Crop biomass was negatively affected by weed levels and positively influenced by soil or crop P concentrations.

• Crop biomass was consistently higher on conventional farms due weeds being controlled through the use of herbicides and soil extractable P being increased through the use of fertilisers containing soluble P.

• Root biomass and the root-shoot ratio were higher on the alternative farms.

• VAM colonisation was not positively correlated with crop biomass, root length, crop P concentration or crop P content, however the actual contribution of VAM fungi to crop growth could not be accurately estimated as no non-VAM controls were available.

• There was no indication that different biological processes were controlling the functioning of conventional and alternative farms.

Chapter Six

Colonisation of Clover by VAM Fungi in Pastures on Mixed Farms

This chapter presents results from the sampling of conventional and alternative annual pastures on mixed farms at Ardlethan and Cootamundra in winter 1995. Soil extractable P concentrations were measured, clover plants examined for VAM colonisation levels and shoot P and N concentrations determined.

6.1. Aims

The aims of this chapter were to investigate the following questions.

- What factors control the level of VAM colonisation in clover plants in mixed farm pastures?

- Will alternative pastures have levels of VAM colonisation significantly higher than the levels in conventional pastures, as found in the crops described in Chapter 5?

6.2. Introduction

A pasture phase is an important part of the rotation on most mixed farms in SE Australia. Pasture improves soil structure and through the actions of legumes, it allows accumulation of soil N (Dalal *et al.* 1995; Grace *et al.* 1995; Murphy and Harte 1992; Peoples *et al.* 1995). Pasture also allows the farmer to diversify production through keeping livestock, generally sheep, which are primarily utilised for wool and fat lamb production. Cattle are often also farmed. Eight pasture paddocks of various ages were sampled on the paired farms at Ardlethan and Cootamundra in early winter 1995. Sampling was intended to include both a first year pasture and an older pasture on each farm, however, owing to the resowing of crops into paddocks where crops had failed due to the 1994 drought, no first year pastures were available.

6.3. Methods

Paddocks were sampled on 21 June at Ardlethan and 7 July at Cootamundra. At Ardlethan, one paddock containing 2nd year pasture was sampled on the conventional III farm (Plate 6.1), while three paddocks were sampled on the organic farm; two paddocks containing 3rd year pasture (i and ii) and one paddock containing 8th year pasture. At Cootamundra on the conventional farm, samples were taken from 5th year pasture and from a paddock which, it was claimed by the farmer, had never been cropped and was therefore considered to be permanent pasture (J. Orgill, pers. comm.; Plate 6.2). On the biodynamic farm at Cootamundra, paddocks which had been under pasture for four and 11 years were sampled (Table 6.1). Paddocks are referred to in this chapter by farm and pasture age (Table 6.1).

The composition of the pasture was visually estimated for each paddock after walking a transect and the occurrence of major species or functional groups, as well as stubble and bare ground, expressed as a proportion of paddock area. Clover plants, *Trifolium subterraneum* L. at Ardlethan and *Trifolium* spp. at Cootamundra, were sampled from 10 sites as described in section 3.2. A number of individual plants were

Plate 6.1. Pasture on the conventional (III) farm at Ardlethan in June 1995. The combined effects of a high yielding crop in 1993 and the 1994 drought have resulted in a large areas of stubble and bare ground. Capeweed (*Arctotheca calendula* (L.) Levyns) is the dominant species.

Plate 6.2. View across the permanent pasture paddock and other paddocks containing crops and pasture on conventional farm at Cootamundra in July 1995. The biodynamic farm can be seen in the far left-hand corner.

Table 6.1. Management details of the pasture paddocks sampled in 1995.

Location	Farm management strategy	Known history (years)	Pasture age	Last fertiliser application	Fertiliser (kg ha^{-1})	Nutrients applied (kg ha^{-1}) P	Others
Ardlethan	Conventional	4 pasture, 1 wheat, 1 pasture	2nd year	1994	single superphosphate 111	10	22 Ca, 12 S
	Organic	5 pasture, 1 wheat, 2 pasture	3rd year (i)	1992	reactive phosphate rock 149	19.5	52 Ca, 2 S
		5 pasture, 2 wheat, 1 pasture	3rd year (ii)	1991	reactive phosphate rock 149	19.5	52 Ca, 2 S
		2 wheat, 7 pasture	8th year	1987	reactive phosphate rock 149	19.5	52 Ca, 2 S
Cootamundra	Conventional	1 pasture, 1 oats, 3 wheat, 4 pasture	5th year	1990	diammonium phosphate 70	14	12.6 N
		never cropped	permanent	?	-	-	-
	Biodynamic	5 pasture, 2 wheat, 2 pasture	4th year	1991	reactive phosphate rock 23	3	8.1 Ca
		2 wheat, 7 pasture	11th year	?	-	-	-

removed from each site and, owing to their small size, bulked together. Roots were examined for colonisation by VAM fungi (§3.5) and the concentration of P and N was measured in the shoots (§3.3). Soil to be assessed for extractable P concentration (Colwell 1963) was sampled to 100 mm from the 10 sites in each paddock, air dried and sent to Wesfarmers CSBP (Perth, Western Australia) for analysis. Soil pH was analysed as described in section 3.6. Rainfall figures were provided by the Bureau of Meteorology. Data are presented as means and s.e.m.s and multiple regressions are also conducted using the statistical package JMP® (§3.9). Owing to the small size of plants, shoot samples from two or three sampling sites in a paddock were sometimes combined for the nutrient analysis. Sites where this occurred were excluded from the statistical analyses presented in Table 6.5.

6.4. Results

Monthly rainfall for the year preceding sampling, June 1994 to July 1995, is presented in Table 6.2. The 1994 drought broke in January 1995 at both locations and reasonably high rainfall also occurred in May, June and July 1995.

Table 6.2. Monthly rainfall at Ardlethan and Cootamundra from July 1994 to July 1995.

	J	A	S	O	N	D	J	F	M	A	M	J	J
Ardlethan	11	15	4	9	32	11	130	4	0	18	96	60	50
Cootamundra	27	12	19	44	42	27	146	4	0	29	134	70	99

Table 6.3 presents estimates of the ground cover composition in each paddock. Clovers were subterranean (*Trifolium subterraneum* L.) at Ardlethan and a mixture of subterranean and other unidentified species at Cootamundra. Annual rye grass (*Lolium rigidum* Gaud.) was a major component of the grasses present on all farms, except on the biodynamic farm where cocksfoot (*Dactylis glomerata* L.) was predominant. Other grasses present included silver grass (*Vulpia brominoides* (L.) S. F. Gray.), barley grass (*Hordeum leporinum* Link) and *Avena*, *Bromus* and *Stipa* species (Derrick 1996). Lucerne (*Medicago sativa* L.) had been sown into most of the paddocks but was well-established only in two. There were high levels of weeds — up to 40% of ground cover — in many of the paddocks particularly common were capeweed (*Arctotheca calendula* (L.) Levyns), Paterson's curse (*Echium plantagineum* L.) and various species of thistles. The conventional paddock at Ardlethan was poorly vegetated with 30% of ground cover as stubble and 30% as bare ground. Overall, pasture composition varied greatly, even between pastures on the same farm.

Table 6.3. Pasture composition, expressed as proportion of paddock area, of paddocks sampled in 1995 from the conventional (Con.) and organic farms at Ardlethan and conventional and biodynamic farms at Cootamundra.

	Ardlethan				Cootamundra			
	Con.	Organic			Conventional		Biodynamic	
	2nd year	3rd year (i)	3rd year (ii)	8th year	5th year	perm.	4th year	11th year
Clover	5	15	30	15	35	40	20	25
Grasses	15	25	60	15	15	30	75	55
Lucerne	-	-	few	-	30	-	-	15
Capeweed	20	10	-	40	-	-	-	-
Paterson's curse	-	40	-	10	10	-	-	-
Thistles	-	-	10	-	10	10	few	few
Other weeds	few	5	few	10	few	20	few	5
Stubble	30	-	-	-	-	-	-	-
Bare ground	30	5	-	10	-	-	5	-

Table 6.4. Colwell soil extractable P, soil pH, concentration of P and N in clover shoots and percentage of clover root length colonised by VAM fungi in paddocks sampled in 1995 from the conventional (Con.) and organic farms at Ardlethan and conventional and biodynamic farms at Cootamundra; mean *(s.e.m.)*, n=10 (soil, VAM), n=3-10 (shoot nutrient concentrations).

	Ardlethan				Cootamundra			
	Con.	Organic			Conventional		Biodynamic	
	2nd year	3rd year (i)	3rd year (ii)	8th year	5th year	perm.	4th year	11th year
Soil extractable P ($\mu g\ g^{-1}$)	17.3 *(2.6)*	9.6 *(0.5)*	11.4 *(0.6)*	7.5 *(0.8)*	74.2 *(4.1)*	34.1 *(6.2)*	17.5 *(1.1)*	15.4 *(1.4)*
Soil pH	6.1	6.6	6.3	6.2	6.0	5.9	5.6	6.1
Shoot N (%)	2.70 *(0.03)*	2.54 *(0.06)*	3.49 *(0.09)*	3.25 *(0.04)*	3.41 *(0.09)*	3.46 *(0.06)*	3.33 *(0.06)*	3.17 *(0.08)*
Shoot P (%)	0.23 *(0.01)*	0.15 *(0.01)*	0.26 *(0.004)*	0.19 *(0.02)*	0.41 *(0.01)*	0.32 *(0.02)*	0.31 *(0.01)*	0.28 *(0.01)*
VAM (%)	61 *(4)*	64 *(4)*	81 *(2)*	77 *(2)*	63 *(4)*	68 *(4)*	75 *(2)*	76 *(3)*

Soil extractable P, soil pH, clover shoot P and N and VAM (%) in each paddock are presented in Table 6.4. Soil extractable P was higher at Cootamundra than Ardlethan and was higher on the conventional farms. Soil pH was similar in all paddocks. Shoot N was similar in all paddocks, except the conventional and organic 3rd year (i) paddocks at Ardlethan where it was much lower, while shoot P was higher at Cootamundra. Thus for the shoot nutrient concentrations, there were no consistent differences between the conventional and alternative farms. The average VAM (%) was high in all paddocks, ranging from 61-81%. Although the alternative farms had a higher level of colonisation, the difference was generally not significant.

Results from the statistical analyses are presented in Table 6.5. Pasture age, soil pH and farm location had no significant effect in any model. Clover VAM (%) was most strongly correlated with the shoot P and N, with P decreasing colonisation and N increasing colonisation (Fig. 6.1.a). There was a strong negative linear relationship between VAM (%) and shoot P when shoot N was > 3.1%. When shoot N was < 3.1%, VAM (%) was lower, however this lower colonisation also corresponded with lower P (Fig. 6.1.a, b). Shoot P and N were highly linearly positively correlated and shoot P was strongly positively correlated with log soil extractable P (Table 6.5 and Fig. 6.1.b, c).

Table 6.5. Results from statistical analysis of pasture data. Parameters included are percentage of clover root length colonised by VAM fungi, Colwell soil extractable P ($\mu g\ g^{-1}$) and clover shoot P and N (%).

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
VAM (%)	full model	-	-	8.1	0.0015	0.30	34
	intercept	26.0	12.5	2.1^t	0.045		
	shoot N	22.6	5.6	16.2	0.0003		
	shoot P	-78.8	24.3	10.5	0.0028		
Shoot N	full model	-	-	67.9	<0.0001	0.66	36
	intercept	2.10	0.13	16.6^t	<0.0001		
	shoot P	3.54	0.43	67.9	<0.0001		
Shoot P	full model	-	-	187.6	<0.0001	0.84	36
	intercept	-0.06	0.03	-2.46^t	0.019		
	log extractable soil P	0.26	0.02	187.6	<0.0001		

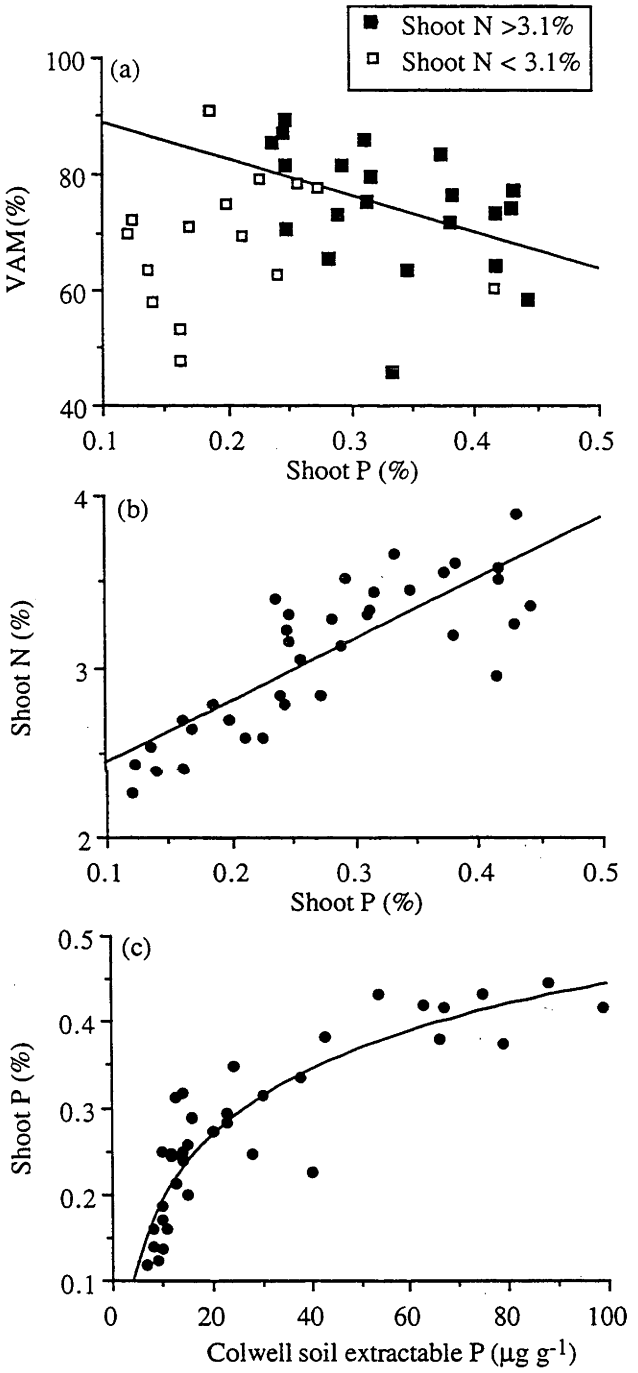


Figure 6.1. Simple regressions of relationships between a) the percentage of clover root length colonised by VAM fungi and shoot P for sites where shoot N was >3.1% (r^2=0.17) and sites where shoot N was <3.1%, b) shoot P and shoot N (r^2=0.67) and c) shoot P and Colwell soil extractable P (r^2=0.80). Data from individual sampling sites in eight pasture paddocks on four mixed farms in 1995.

6.5. Discussion

6.5.a. Soil and Shoot Nutrient Concentrations

Soil extractable P was higher on the conventional farms and was strongly correlated with shoot P. At Cootamundra, shoot P and N were higher on the conventional farm. However, at Ardlethan this was not the case due to the poor state of the one conventional pasture paddock (Plate 6.1 and Table 6.3; discussed further below).

A gauge of the nutritional status of the pastures was provided by clover shoot P and N. Normal and deficient P concentrations for subterranean clover leaves and petioles are 0.25-0.50% and <0.20% respectively (Weir and Cresswell 1994; see also Pinkerton and Randall 1994). The average shoot P in clover at Cootamundra was in the normal range. However at Ardlethan, shoot P in the conventional paddock was less than normal and in two of the organic paddocks, shoot P was deficient (0.15 and 0.19%; Table 6.4).

Normal and low N for subterranean clover leaves and petioles are 3.3-5.5% and 3.0-3.2% respectively (Weir and Cresswell 1994). At Cootamundra, most paddocks were within the normal limits, however the older pasture on the biodynamic farm had low shoot N. At Ardlethan, clover in the oldest pasture paddock on the organic farm had low shoot N, while clover in the 3rd year (i) pasture was deficient (2.5%; Weir and Cresswell 1994). Clover in the conventional paddock was also deficient (2.7%).

The paddocks where clover shoots were deficient in N had supported high yielding crops in 1993 on the conventional farm and 1992 on the organic farm. Both these crops had produced large amounts of stubble, largely decomposed on the organic paddock by 1995, which had not been burned. In combination with the 1994 drought, this had resulted in large areas of bare ground in the conventional paddock (Plate 6.1) and low levels of clover in both paddocks (Table 6.3). Compared with the 3rd year (ii) paddock on the organic farm, the paddock with deficient N concentrations had less clover present and a very high proportion of ground cover, 40%, consisting of the weed Paterson's curse.

6.5.b. VAM Colonisation

Owing to the 1994 drought and the annual nature of the pastures, all plants sampled were assumed to have germinated and grown since the breaking of the drought in January 1995. Therefore, the high VAM (%) in all paddocks indicated that the drought had not reduced the inoculum levels of soil in pasture paddocks sufficiently to significantly impact on colonisation levels, even though these paddocks — particularly at Ardlethan — were virtually devoid of vegetation during the drought.

The negative correlation between plant P and VAM (%) was consistent with the results from the crops on these mixed farms (Fig. 5.12). The positive correlation

between plant N and VAM (%) appeared largely due to the lower colonisation at those sites where both N and P were low (Fig. 6.1.a). Low Colwell extractable P, 0.62 $\mu g\ g^{-1}$ was found to restrict VAM colonisation by Bolan *et al.* (1984), while Vejsadová *et al.* (1989) reported that VAM colonisation was restricted at deficient N concentrations. It is not clear in this instance whether low P or low N, or a combination of both, were responsible for the low VAM (%) at some sites.

However, overall, clover VAM (%) was high (> 60%) in all paddocks. Similar levels of colonisation were found by Ryan (1992) in first year pasture on the conventional I (66%) and organic farms (79%) at Ardlethan in October 1991. This indicates that VAM colonisation quickly returns to a high level after a paddock is returned to pasture after cropping. The higher soil extractable P on the conventional farms was not sufficient to markedly reduce VAM colonisation. Therefore, assuming that production of VAM inoculum will generally reflect colonisation levels, the level of VAM inoculum in paddocks entering a first year of cropping is likely to be high on both conventional and alternative farms. Thus, any beneficial effects from VAM hyphae on soil structure (Gatehouse 1995; Miller and Jastrow 1990; Tisdall and Oades 1979) will be present in pastures under both conventional and alternative management.

6.6. Conclusions about the Mixed Farm Pastures

- Soil extractable P was higher on the conventional farms and was strongly positively correlated with clover shoot P.
- Clover plants in the conventional and some organic pastures at Ardlethan were deficient in P and N.
- Clover from both conventional and alternative farms was highly colonised by VAM fungi, with > 60% of root length colonised.
- VAM colonisation levels in clover were slightly higher on the alternative farms.
- Both shoot P and N were affecting VAM colonisation levels, with high P and low N and/or perhaps low P restricting colonisation.
- It is likely that VAM inoculum accumulates under pasture and is high when both conventional and alternative paddocks enter a first year of cropping.

Chapter Seven

Glasshouse Trials Examining VAM Colonisation and Plant Growth in Soils from Mixed Farms

This chapter presents the results from two glasshouse trials designed to assess the contribution of VAM fungi to wheat growth in soil collected from the organic mixed farm at Ardlethan. In the first trial, wheat grown in field soil and soil sterilised using either heat or gamma irradiation to eliminate VAM fungi, was compared with treatments where VAM inoculum was added. Low light levels in the glasshouse appeared to significantly influence the outcome of this trial. In the second trial, wheat was grown in irradiated soil, with and without addition of VAM inoculum, P and Zn. Two factors which may effect the relevance of glasshouse trials to field conditions were also varied; VAM species and plant density.

7.1. Introduction

The contribution of VAM fungi to plant growth is difficult to assess in field studies, such as those presented in Chapters 5 and 6, due to the lack of non-VAM controls and the complicated relationships between plant growth, plant P concentration and VAM colonisation levels (§1.5). Sterilisation of soil in the field was not possible using the resources available for this project and, furthermore, infringed the codes of practice of alternative farms. Thus the contribution of VAM fungi to wheat growth on the red earth soils found on the mixed farms sampled in this project — a soil type typical of the SE Australian wheatbelt — was assessed using glasshouse trials. Wheat was grown in sterilised soil and comparisons made between plants grown with, and without, addition of VAM inoculum. However, the results from such trials may not reflect the situation in the field due to differences in factors such as microclimate, plant density, VAM species, soil micro-organisms and pathogen activity (§1.5 and §12.1.b.ii). The two glasshouse trials presented in this chapter were designed to minimise the differences in these factors between the inoculated and non-inoculated treatments. Moreover, the trials assessed the effect of some of the above factors on the relationship between VAM fungi and plant growth, allowing conclusions to be drawn about the relevance of the trials to field conditions.

The first glasshouse trial compared two soil sterilisation techniques, irradiation and heat sterilisation, as sterilisation techniques may differ in their effects on concentrations of soil extractable nutrients and soil micro-organisms. This trial was conducted during winter and, unintentionally, became an examination of the effect of VAM fungi on wheat (and clover) growth under low light conditions, as maximum daily light levels in the glasshouse were ≤50% of those outside. The second trial was conducted over summer under much higher light conditions and included a larger number of treatments. Phosphorus and Zn treatments were included as Thompson (1990), in a glasshouse trial using an Australian vertisol soil, found uptake of these nutrients to be enhanced by VAM fungi. The P treatment also acted as a simulation of the fertiliser practices used on the conventional mixed farms described in Chapter 5. Three types of VAM inoculum — field soil and inoculum from pure cultures of *Glomus intraradices* and *Scutellospora calospora* — were used to compare the effects of indigenous VAM fungi with two species commonly used in glasshouse trials by researchers. As plant density is a factor that may affect the relevance of glasshouse trials to field conditions (Bååth and Hayman 1984), two plant densities were included in the trial.

7.2. Trial One: A Comparison of Soil Sterilisation Methods

7.2.a. Aims

The aims of the trial were to address the following questions.

- Do heat sterilisation and gamma irradiation result in VAM fungi being eliminated from soil?
- Does the method of soil sterilisation influence plant growth?
- What effects do VAM fungi have on growth of wheat (*Triticum aestivum* L.)?
- What effects do VAM fungi have on growth of subterranean clover (*Trifolium subterraneum* L.)?

7.2.b. Methods

Soil was collected from a recently tilled fallow paddock on the organic farm at Ardlethan in February 1996 and passed through a 10 mm sieve. Colwell extractable P was assessed on a subsample of soil by Wesfarmers, CSBP (Perth, Western Australia). The experiment was a fully crossed factorial design with two factors.

1) *VAM.* The two treatments were no added VAM inoculum '- inoculum' and added inoculum '+ inoculum'. Inoculum was produced by collecting soil from the organic farm at Ardlethan in December 1995 and growing wheat through to senescence in a glasshouse. The soil and wheat roots were air dried and stored in bins in the glasshouse. In a preliminary trial, wheat grown in this soil had 60% of root length colonised by VAM fungi after four weeks. In the inoculated pots, 400 g of field soil was removed from the top 1 kg of soil and replaced with 400 g of inoculum.

2) *Soil treatment.* The three soil treatments were untreated field soil 'field soil', heat sterilised soil 'heat sterilised' and irradiated soil 'irradiated'. The heat sterilised soil was first moistened before being heated at 100°C for three hours. The gamma irradiated soil was treated at 25 kGy (§3.7).

Treatments were replicated five times, each set of replicates forming a randomised block in the glasshouse. Two kilograms of air-dried soil were used to fill standard 175 mm pots. Sterilised gravel was placed in the bottom of pots to improve drainage. To avoid toxic effects from the sterilisation, pots were heavily watered and left for one week before planting and 80 ml of filtrate was added to all pots at planting (§3.7). Wheat seedlings cv. Banks were planted in a 1:1 vermiculite:perlite mix and three seedlings, at the two leaf stage, transplanted into each pot in late June, 1996. Pots were weeded and watered by hand. Basal nutrients, minus P, were applied at planting and subsequently every two weeks (§3.7). Maximum daily glasshouse light levels were

low, varying from 200 to 400 μmol m^{-2} s^{-1}.

One week after transplanting, leaf area in each pot was calculated by measuring the length and width of each leaf and applying the formula; *area = length x width x 0.7* (De Marco 1990). Leaf area measures were converted to dry weights using formula; *dry mass = 0.005172 x leaf area.* All plants were harvested at week 7, when the wheat began to head. VAM (%), shoot dry weight and root wet weight in each pot were measured as described in Chapter 3 (§3.3, §3.4 and §3.5). Root wet weights were converted to dry weight equivalents (§3.4.b). Shoot relative growth rates (RGR) were calculated as *RGR = (log(harvest shoot weight) - log(week 1 shoot weight))/7.*

Results were analysed using the statistical package JMP® (§3.9). Parameters fitted in ANOVAs were block (1-5), soil (field, heat sterilised, irradiated) and inoculum (- inoculum, + inoculum). These parameters were used as, even though the field soil was not sterile and caused VAM colonisation, the addition of inoculum increased this colonisation, resulting in changes in plant growth similar to those exhibited when VAM fungi were inoculated into the two sterilised soil treatments. The effects of VAM colonisation levels, irrespective of inoculum addition, were assessed through ANCOVAs using the parameters block (1-5), soil (field, heat sterilised, irradiated) and the continuous variable, VAM (%).

An additional experiment was conducted using subterranean clover cv. Woolgenellup. Pots were filled either with 800 g of irradiated soil or 600 g of irradiated soil and 200 g of inoculum. All pots were treated with basal nutrients and filtrate, as described above, and were also inoculated with a mixture of five strains of *Rhizobia* bacteria suitable for subterranean clover. Two clover plants were transplanted into each pot and each treatment replicated four times. Plants were harvested at week 7 and VAM (%), shoot dry weight and root wet weight in each pot measured as described in Chapter 3 (§3.3, §3.4 and §3.5). Root wet weights were converted to dry weight equivalents (§3.4.b).

7.2.c. Results

Colwell soil extractable P was 13 μg g^{-1}. The results from ANOVAs examining the influence of soil treatment and inoculum addition on VAM colonisation and wheat growth are presented in Table 7.1 and shown as interaction graphs in Figure 7.1. There was no VAM colonisation in the non-inoculated heat sterilised or irradiated soil, while wheat in the field soil had around 30% of root length colonised. Addition of inoculum increased VAM colonisation in all treatments, but to a greater extent in the sterilised soil treatments (Fig. 7.1.a).

Plate 7.1. Wheat growing in three treatments in the glasshouse trial: non-sterile field soil (Field); irradiated soil reinoculated with VAM fungi (+ VAM); and irradiated soil with no VAM fungi (-VAM). Note that all plants look healthy.

Table 7.1. Results from ANOVAs of the percentage of root length colonised by VAM fungi at harvest, shoot dry weight at week 1 (g pot^{-1}), shoot dry weight at harvest (g pot^{-1}), root dry weight at harvest (g pot^{-1}), the root-shoot ratio at harvest and the relative growth rate (RGR) between week 1 and harvest. Parameters included are block (1-5), soil treatment (field, heat sterilised, irradiated) and VAM inoculum (- inoculum, + inoculum). All significant interaction terms are included.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	23.3	<0.0001	0.87	30
	block	2.8	0.05		
	soil treatment	33.8	<0.0001		
	inoculum	110.3	<0.0001		
	soil x inoculum	10.4	0.0008		
Shoot dry weight (week 1)	full model	5.0	0.002	0.49	30
	block	0.6	0.6		
	soil treatment	11.8	0.0003		
	inoculum	8.8	0.007		
Shoot dry weight (harvest)	full model	19.1	<0.0001	0.81	30
	block	11.0	<0.0001		
	soil treatment	22.2	<0.0001		
	inoculum	44.9	<0.0001		
Root dry weight (harvest)	full model	9.4	<0.0001	0.67	30
	block	7.6	0.0005		
	soil treatment	3.0	0.07		
	inoculum	29.0	<0.0001		
RGR	full model	2.4	0.05	0.31	30
	block	1.2	0.3		
	soil treatment	3.0	0.07		
	inoculum	2.3	0.1		
	soil x inoculum	4.5	0.02		
Relative growth rate (week 1-7)	full model	26.3	<0.0001	0.86	30
	block	33.8	<0.0001		
	soil treatment	12.0	0.0003		
	inoculum	24.7	0.0001		

Shoot dry weight at week 1 was significantly affected by soil treatment, being greater in the irradiated soil. Addition of inoculum resulted in a decrease in shoot dry weight (Fig. 7.1.b and Plate 7.1). The same trends were evident at harvest, with the addition of inoculum reducing shoot weight by, on average, 23%. Root dry weight at harvest was significantly influenced only by the addition of inoculum which decreased biomass by 26% (Fig. 7.1.d). The root-shoot ratio decreased in response to inoculum addition in the field and heat sterilised soil, but increased in the irradiated soil. The shoot RGR between week 1 and harvest was lower in the field soil than the two sterilised soil treatments and decreased with addition of inoculum.

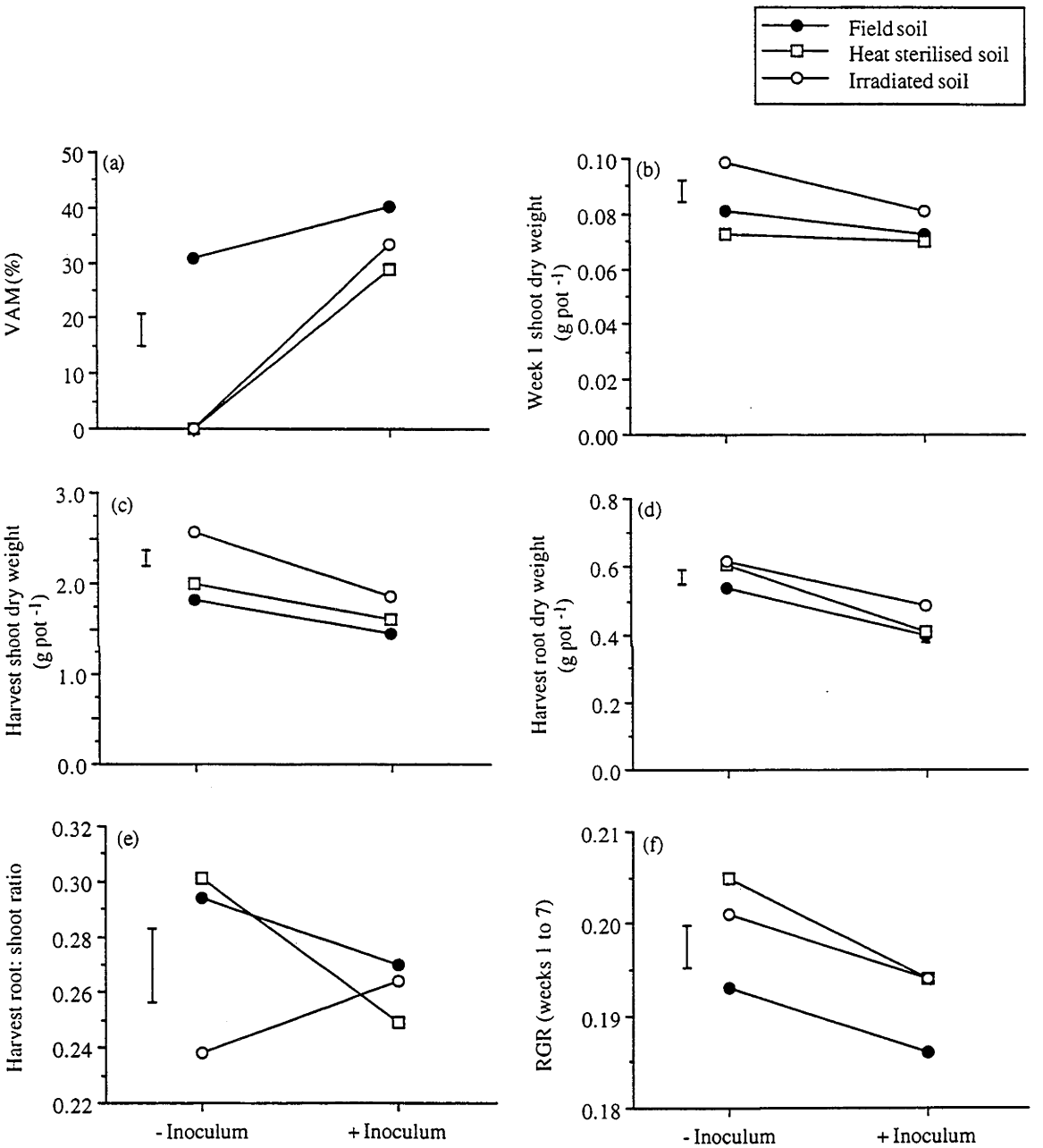


Figure 7.1. Interactions between VAM inoculum (absent, present) and soil treatment (field, heat sterilised, irradiated) for a) percentage of root length colonised by VAM fungi at harvest (week 7), b) shoot dry weight at week 1, c) shoot dry weight at harvest, d) root dry weight at harvest, e) the root-shoot ratio at harvest and f) the shoot relative growth rate (RGR) from week 1 to harvest; estimated means and LSD at p=0.05.

To further explore the effect VAM colonisation on plant growth, the analyses shown in Table 7.1 were repeated as ANCOVAs with inoculum (-, +) replaced with the continuous variable, VAM (%). VAM colonisation correlated negatively with shoot dry weight, root dry weight and the RGR. The effect of VAM colonisation varied with soil treatment for the root-shoot ratio (Table 7.2).

Table 7.2. Results from ANCOVAs of shoot dry weight at harvest (g pot^{-1}), root dry weight at harvest (g pot^{-1}), the root-shoot ratio and the relative growth rate (RGR) between week 1 and harvest. Parameters included are block (1-5), soil (field, heat sterilised, irradiated) and the percentage of root length colonised by VAM fungi. All significant interaction terms are included.

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio	Prob.	r^2	n
Shoot dry weight	full model	-	-	11.7	<0.0001	0.72	30
	block	-	-	5.0	0.005		
	soil treatment	-	-	6.1	0.008		
	VAM (%)	-0.015	0.003	11.7	<0.0001		
Root dry weight	full model	-	-	6.0	0.0006	0.54	30
	block	-	-	3.8	0.02		
	soil treatment	-	-	0.7	0.5		
	VAM (%)	-0.005	0.001	15.1	0.0008		
Root-shoot ratio	full model	-	-	2.4	0.05	0.30	30
	block	-	-	0.8	0.52		
	soil treatment	-	-	5.9	0.009		
	VAM (%)	-0.0001	0.0007	0.1	0.8		
	soil x VAM (%)	-	-	4.8	0.02		
RGR	full model	-	-	21.6	<0.0001	0.83	30
	block	-	-	23.3	<0.0001		
	soil treatment	-	-	1.6	0.2		
	VAM (%)	-0.0003	0.00007	17.4	0.0004		

Graphs of the three models in Table 7.2 where VAM colonisation had a consistent effect are presented in Figure 7.2. The predicted values of the plant growth measures from the ANCOVA models in Table 7.2 were used, as this allowed the effect of VAM colonisation to be clearly observed, as the data had been adjusted for the effects of soil treatment and block. There was a strong negative non-linear relationship between VAM (%) and shoot dry weight, root dry weight and RGR.

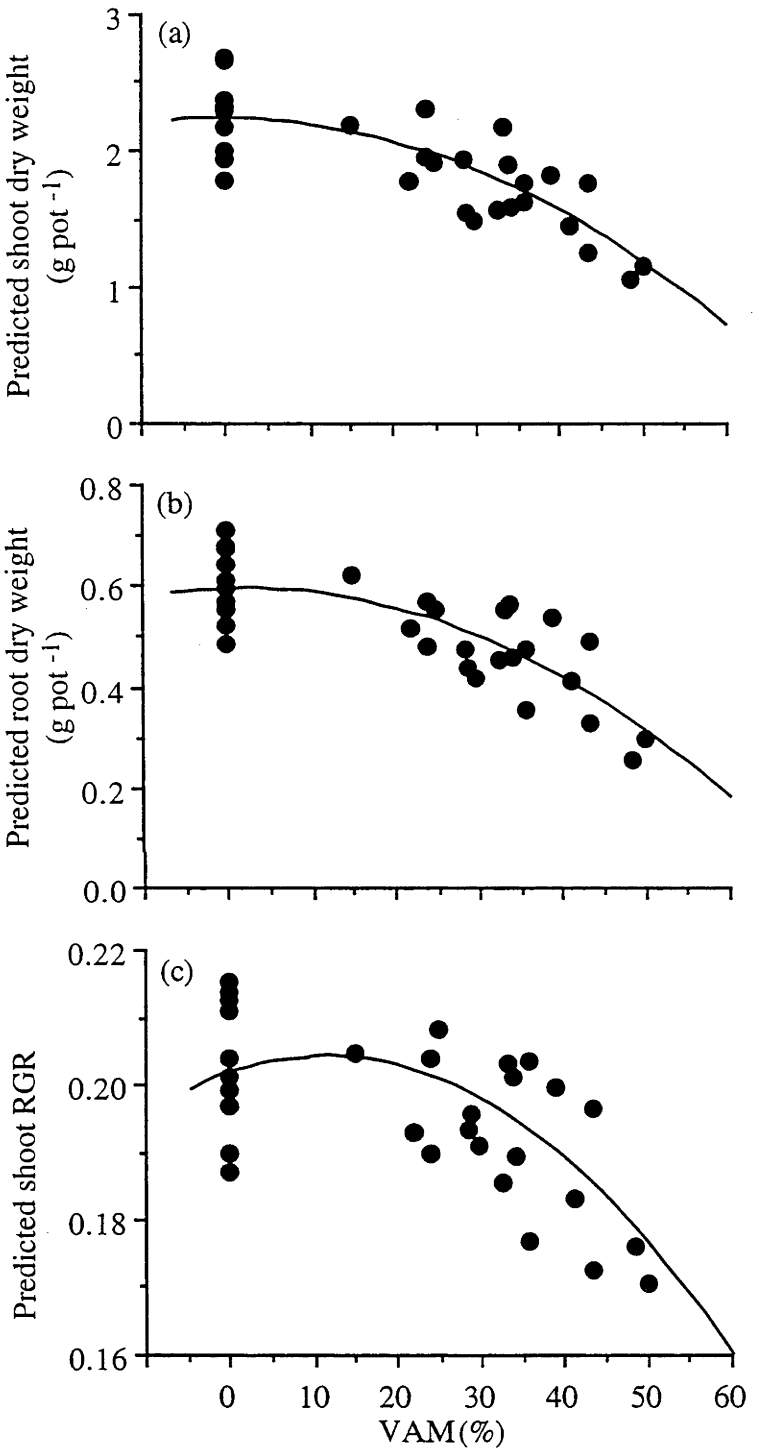


Figure 7.2. Relationships between the percentage of root length colonised by VAM fungi at harvest and the predicted values, that is, the values adjusted for the effects of block and soil treatment, from the ANCOVAs in Table 7.2 for a) shoot dry weight at harvest ($y=0.604 - 0.00011x - 0.00012x^2$; $r^2=0.64$), b) root dry weight at harvest ($y=2.253 - 0.0025x - 0.00038x^2$; $r^2=0.65$) and c) the relative growth rate (RGR) between week 1 and harvest ($y=0.203 + 0.00024x - 0.000016x^2$; $r^2=0.46$).

Results from the clover plants are presented in Table 7.3. Colonisation by VAM fungi resulted in a significant increase in shoot growth (60%), an increase in root weight (34%) and a significant decrease in the root-shoot ratio.

Table 7.3. Percentage of root length colonised by VAM fungi, shoot dry weight and root dry weight of clover plants grown in irradiated soil with and without addition of VAM inoculum; mean *(s.e.m.)*, n=4.

	VAM (%)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root-shoot ratio
- inoculum	0	0.43 *(0.07)*	0.32 *(0.06)*	0.75 *(0.03)*
+ inoculum	19.0 *(1.7)*	0.69 *(0.04)*	0.43 *(0.05)*	0.62 *(0.04)*

7.2.d. Discussion

(i) Soil Sterilisation

Both methods of soil sterilisation effectively eliminated VAM fungi from the soil. Plants grown in the irradiated soil were significantly larger than plants in the field soil or heat sterilised soil, both at week 1 and at harvest. Sterilisation is likely to result in increased concentrations of soluble N and extractable P in soil (Thompson 1990). This may have occurred to a greater degree in the irradiated soil, although basal nutrients, containing N, were applied to all pots and irradiation was found to have little effect on soil total N and soil extractable P in section 7.3. Alternatively, the lower growth in the heat sterilised treatment may have been due to release of toxic compounds and the lower growth in the field treatment due to higher VAM colonisation levels (see §7.2.c.iii).

However, this trial was designed to allow comparisons between plants in sterilised soil and plants grown in sterilised soil with added inoculum. Thus, slight differences in plant growth due to soil sterilisation method do not effect the conclusions drawn about the influence of VAM fungi on plant growth.

(ii) VAM Colonisation Levels in Wheat

The addition of inoculum resulted in VAM colonisation levels >15% in all pots. Moreover, it increased VAM colonisation in the field soil from 32% to 40%, indicating that inoculum levels in the field soil were not adequate for a maximum rate of VAM colonisation. However, the situation in the glasshouse will differ from the field where wheat growth is slower and the rate at which VAM fungi spread throughout the root system better able to match root growth (see §5.4.b.ii).

(iii) Wheat Growth

The introduction of VAM inoculum decreased the root and shoot biomass (Fig. 7.1). Two factors may have been responsible, introduction of soil pathogens — see (Thompson 1990) — or colonisation by VAM fungi. The introduction of a pathogen seems unlikely to have reduced wheat growth as filtrate from the soil used as inoculum was added to all pots and all plants in the trial appeared healthy and disease-free (Plate 7.1). No fungi, other than VAM fungi, were observed colonising inside the roots. Moreover, the strong negative relationship between VAM (%) and plant growth (Fig. 7.2) suggests VAM fungi were responsible. It seems unlikely that the effects of an internal fungal pathogen would have increased as VAM colonisation increased (Newsham *et al.* 1995; Thompson and Wildermuth 1989). However, the interactions between VAM fungi and soil pathogens are complex and varied (Linderman 1992), and the presence of a pathogen on the root surface — or a bacterial or viral pathogen — cannot be completely ruled out. The use of single VAM species cultures as inoculum, as occurred in the second trial presented in this chapter, should reduce the likelihood of pathogens being introduced.

Thus, it appears that colonisation by VAM fungi reduced wheat growth. This result is contrary to that of Thompson (1990) and the results from the second glasshouse trial (§7.3). It is likely that low light levels in the glasshouse — maximum daily levels of 200-400 $\mu mol\ m^{-2}\ s^{-1}$, compared with 1100 $\mu mol\ m^{-2}\ s^{-1}$ outside— were responsible (Daft and El-Giahmi 1978; Olsen *et al.* 1996; Son and Smith 1988), perhaps in combination with short winter day lengths. Smith and Gianinazzi-Pearson (1990) examined growth of onions, with and without VAM fungi, under two levels of irradiance, 190 and 410 $\mu mol\ m^{-2}\ s^{-1}$. At the lower light level, plant growth was significantly reduced and the large positive effect of VAM colonisation seen at the higher light level, virtually eliminated. Presumably, at low light levels the rate of host plant photosynthesis is reduced, plant growth becomes carbon limited and the carbon used by the VAM fungi is at the expense of plant growth (Son and Smith 1988). Consequently, relationships — such as those shown in Figure 7.2 — with the negative effect on plant growth increasing as VAM colonisation increases, would be expected.

Wheat has a fine-branched, extensive root system and is often considered to not be very dependant on VAM fungi (§1.3.d, §1.4.a and §12.1.c). Consequently, a small change in the conditions that allow the symbiosis to be profitable to the host plant may result in a neutral or parasitic relationship. For instance, changes in weather conditions (Manske 1989), or soil conditions; especially increases in extractable P (Mohammad *et al.* 1995). Figure 7.2 also indicated that only after >25% of root length was colonised did the VAM fungi began to reduce plant growth. If light levels had been further reduced, this point of changeover between mutualism and parasitism presumably would have occurred at a lower VAM colonisation level. The

circumstances under which VAM fungi act as parasites is further discussed in section 12.1.c.ii.

The negative effect from inoculum addition on shoot growth at week 1 in the irradiated soil was unexpected, as VAM colonisation levels would have only been low at this time; this could indicate non-random selection of the seedlings which were transplanted into the pots. However, as well as reducing root and shoot biomass, VAM colonisation also caused a reduction in the shoot RGR (Figs. 7.1.f and 7.2.c). Derrick and Ryan (1998) grew wheat seedlings from seed differing in P content, resulting in seedlings of differing weights at week 1, and an identical RGR in all seedlings between weeks 1 and 3 maintained these differences. Indeed, for well-spaced plants, such initial small differences in growth may be maintained until harvest (Black 1957). Thus the lower RGR in the inoculated treatments indicates that the differences in shoot biomass present at week 1 were not solely responsible for the differences at harvest, suggesting that VAM fungi were acting parasitically.

The root-shoot ratio results were inconsistent. The addition of inoculum decreased the ratio in the field soil and the heat sterilised soil, but increased the ratio for the irradiated soil, due to a low ratio in the non-inoculated treatment. There seems no obvious explanation for this difference. Smith and Gianinazzi-Pearson (1990) found that VAM colonisation reduced the root-shoot ratio at light levels of both 190 and 410 $\mu mol\ m^{-2}\ s^{-1}$.

(iv) Clover Growth

Addition of inoculum to clover plants increased the shoot and root dry weight and decreased the root-shoot ratio. These are the expected responses to inoculating clover with VAM fungi in a soil with a low concentration of extractable P (Oliver *et al.,* 1993, unpublished data, in Smith and Read 1997; Schweiger *et al.* 1995; see also §12.1.b.i). Although the clover were grown under the same low light conditions as the wheat, they still exhibited a positive growth response to VAM inoculation. Clover is generally considered more dependent than wheat on VAM fungi, due to its coarser root system and the high P requirements for nodulation (§2.2.d, §2.4.a and §12.1.c; Kucey and Paul 1982; Schweiger *et al.* 1995) and would, therefore, remain P-limited for longer than wheat as light levels decrease. This result also suggests that if a pathogen was decreasing wheat growth in the inoculated treatments, it was specific to wheat.

7.3. Trial Two: The Effects of P, Zn, VAM Species and Plant Density

7.3.a. Aims

The aims of the glasshouse trial were to address the following questions under adequate light conditions.

- What effects do P and Zn additions and plant density have on VAM colonisation levels?
- Are P or Zn limiting wheat growth?
- What contribution do VAM fungi make to wheat growth?
    - Does this contribution change when P and Zn are applied?
        - Does this change if the P is applied after the plant and VAM colonisation are established?
    - Does this contribution vary with the species of VAM fungi present?
    - Does this contribution differ between high and low plant densities?
- What conclusions can be drawn about the relevance of such glasshouse trials to field conditions?

7.3.b. Methods

Soil was collected from under a first year wheat crop on the organic farm at Ardlethan in early October 1996, passed through a 10 mm sieve and bulked. Soil was gamma irradiated at 25 kGy (§3.7). Colwell extractable P, total N and pH were assessed on a subsample of irradiated and non-irradiated soil by Wesfarmers, CSBP (Perth, Western Australia). The experiment was a fully crossed factorial design with four factors.

1) *Zinc*. The two Zn treatments were no added Zn '-Zn' and 15 mg kg^{-1} of Zn as $ZnSO_4.7H_2O$ added to the dry soil as a solution and, after drying, thoroughly mixed through the soil before it was placed in pots '+Zn'.

2) *Density*. The two density treatments were two 'low' or eight 'high' wheat plants in each pot.

3) *VAM*. The four VAM treatments were no colonisation 'control', VAM fungi indigenous to the soil 'field', *Glomus intraradices* or *Scutellospora calospora*. The indigenous VAM fungi were introduced through mixing 8 g of non-irradiated soil and approximately 300 mm of dry roots, collected from under an organic wheat crop in October 1996, into each planting hole. *Glomus* and *Scutellospora* were introduced by mixing 5 g of inoculum — sand, roots and spores — into each planting hole. Inoculum was provided by Dr Sally Smith, Waite Institute, Adelaide.

4) *Phosphorus*. The three P treatments were no added P '-P', 50 mg kg^{-1} of P as $NaH_2PO_4.H_2O$ added to the dry soil as a solution and, after drying, thoroughly mixed through the soil before it was placed in pots '+P', or 50 mg kg^{-1} of P as $NaH_2PO_4.H_2O$ added to the surface of the soil two weeks after seedlings were transplanted into pots 'P-late'.

All treatments were replicated four times, each set of replicates forming a randomised block in the glasshouse. Standard 150 mm pots were filled with 1.2 kg of sieved, irradiated air-dried soil. Sterilised gravel was placed in the bottom of pots to improve drainage. To avoid toxic effects from the sterilisation, pots were heavily watered and left for one week before planting and 80 ml of filtrate was added to all pots at planting (§3.7). Wheat seedlings cv. Banks were planted in a 1:1 vermiculite:perlite mix and seedlings at the two leaf stage were transplanted into pots in early November 1996. Pots were weeded and watered by hand. Basal nutrients, minus P and Zn, were applied every two weeks (§3.7). Maximum daily glasshouse light levels varied from 600 to 1400 $\mu mol\ m^{-2}\ s^{-1}$.

Three weeks after transplanting, leaf width and length were measured on the largest leaf of two randomly chosen plants in each pot. These measures were converted to leaf area as in trial 1. All plants were harvested after 11 weeks, before the grain began to fall out of the heads. Shoot dry weight and root wet weight in each pot were measured and root wet weights converted to dry weights (§3.3 and §3.4). All root samples were washed carefully before staining for VAM colonisation (§3.5), however it was not possible to completely remove all dead roots which were adhering to the roots of the plants grown in the trial. Thus, when the non-VAM controls were checked for VAM colonisation, VAM fungi in some roots from the field crop were staining, even though the fungi, presumably, were dead. Thus for the non-VAM controls, samples were considered to be uncolonised — even when up to 5% of root length stained positively for VAM fungi — providing all colonisation appeared old, that is, did not consist of newly formed entry points or arbuscules. Using these criteria, all controls were found to be uncolonised. This problem was not encountered in the first trial, as soil was collected from a paddock that had been under fallow for three months and contained few roots.

Results were analysed by ANOVA or ANCOVA using the statistical package JMP® (§3.9). Some measures of plant growth were first log transformed. Parameters fitted were Zn (-Zn, +Zn), plant density (low, high), VAM colonisation (control, field, *Glomus*, *Scutellospora*) and P (-P, P-late, +P). Owing to a mishap during the drying of shoots, treatments where Zn and P-late were present could not be included in the analysis. One outlier was removed from the root weight data.

7.3.c. Results

Prior to irradiation, the soil contained 12 μg g^{-1} of Colwell extractable P, 1400 μg g^{-1} of total N and had a pH of 6.2. The irradiated soil contained 14 μg g^{-1} of Colwell extractable P, 1300 μg g^{-1} of total N and had a pH of 6.5. Thus irradiation did not markedly affect these soil properties.

Results from ANOVAs of VAM (%) are presented in Table 7.4 and Figure 7.3. Zinc addition and plant density did not affect VAM colonisation. Plants inoculated with *Glomus* reached a high colonisation level, up to 60%, while *Scutellospora* caused around 30% colonisation. The field soil resulted in variable, but generally very low, levels of colonisation (0-20%). The addition of P markedly reduced VAM colonisation, with P-late resulting in colonisation levels slightly higher than when P was added prior to transplanting. There was a significant interaction between VAM and P, with the addition of P halving colonisation in the *Glomus* treatment, but virtually eliminating colonisation in the field and *Scutellospora* treatments. When the weight of colonised roots was substituted for VAM (%) in the model in Table 7.4, the outcome was identical, except density became significant due to its effect on root biomass (Table 7.6).

Table 7.4. Results from ANOVAs of the percentage of root length colonised by VAM fungi and the weight of roots colonised by VAM fungi (g $plant^{-1}$). Parameters included are block (1-4), Zn addition (-Zn, +Zn), density (low, high), VAM (field, *Glomus*, *Scutellospora*) and P addition (-P, P-late, +P). Non-VAM controls were not included in this analysis. All significant interactions are shown.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	46.0	<0.0001	0.83	118
	block	0.7	0.6		
	Zn	1.3	0.3		
	density	0.7	0.4		
	VAM	102.5	<0.0001		
	P	131.8	<0.0001		
	VAM x P	17.9	<0.0001		
Log dry weight of roots colonised by VAM fungi	full model	13.1	<0.0001	0.57	118
	block	0.8	0.5		
	Zn	0.01	0.8		
	density	10.6	<0.002		
	VAM	51.6	<0.0001		
	P	18.8	<0.0001		
	VAM x P	3.5	0.01		

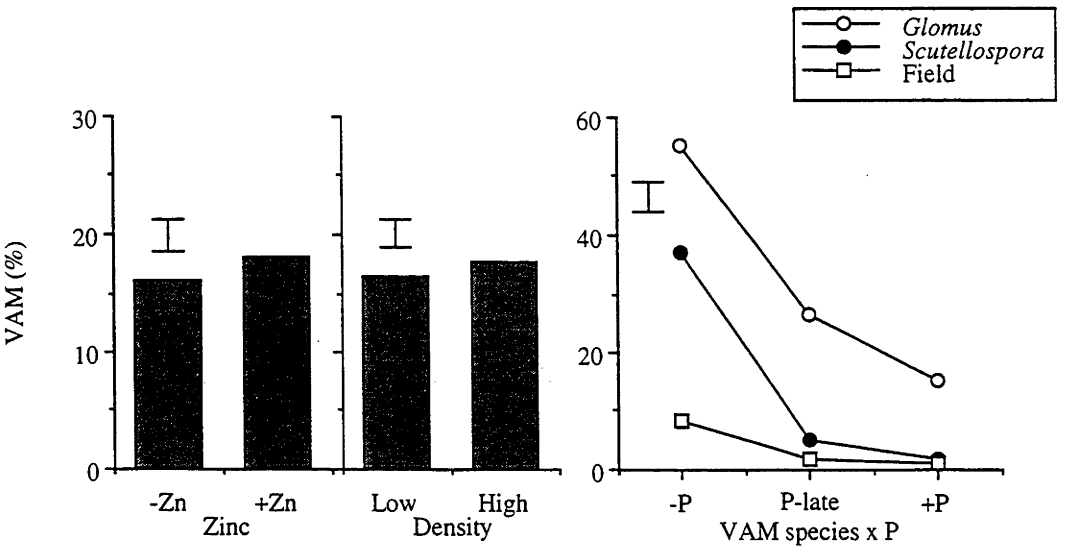


Figure 7.3. Estimated means and LSD at p=0.05 for the percentage of root length colonised by VAM fungi. All significant interaction terms are shown. Note the different scale used on the interaction term graph.

Table 7.5 contains the results from ANOVAs on measures of shoot growth at week 3 and at harvest. Block was a significant influence on all plant growth parameters, indicating a strong environmental gradient in the glasshouse, apparently caused by different levels of shade and proximity to air conditioning vents. The area of the largest two leaves per pot at week 3 was not affected by VAM or Zn addition. Phosphorus addition increased leaf area, although there was no effect from the P-late treatment, which had been applied at week 2. Plants growing at low density had a greater leaf area than plants at high density indicating that competitive effects were already greater in the high density pots (Fig. 7.4.a).

Table 7.5. Results from ANOVAs and an ANCOVA of various measures of wheat shoot growth: the area of the largest two leaves at week 3 (mm^2), the total weight of shoots at harvest (g pot^{-1} and g $plant^{-1}$) and the weight of the heads at harvest (g $plant^{-1}$). Parameters included are block (1-4), Zn addition (-Zn, +Zn), density (low, high), VAM (control, field, *Glomus*, *Scutellospora*) and P addition (-P, P-late, +P). Shoot weight at harvest (g $plant^{-1}$) was also modelled using the continuous measure VAM (%); the coefficient for VAM (%) was 0.0009 ± 0.0004 (se). There were no significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Leaf area (week 3)	full model	30.9	<0.0001	0.66	158
	block	28.9	<0.0001		
	Zn	0.8	0.4		
	density	30.8	<0.0001		
	VAM	1.3	0.3		
	P	91.1	<0.0001		
Total shoot dry weight (pot^{-1})	full model	32.3	<0.0001	0.67	158
	block	28.2	<0.0001		
	Zn	0.3	0.6		
	density	17.1	0.0001		
	VAM	2.7	0.046		
	P	106.9	<0.0001		
Log total shoot dry weight ($plant^{-1}$)	full model	199.7	<0.0001	0.93	158
	block	22.8	<0.0001		
	Zn	0.3	0.6		
	density	1733.1	<0.0001		
	VAM	2.5	0.06		
	P	94.6	<0.0001		
Log total shoot dry weight ($plant^{-1}$)	full model	249.6	<0.0001	0.93	158
	block	23.2	<0.0001		
	Zn	0.2	0.6		
	density	1724.7	<0.0001		
	VAM (%)	5.7	0.02		
	P	85.9	<0.0001		
Log heads dry weight ($plant^{-1}$)	full model	132.4	<0.0001	0.89	159
	block	13.2	<0.0001		
	Zn	0.09	0.8		
	density	1161.2	<0.0001		
	VAM	1.5	0.22		
	P	55.2	<0.0001		

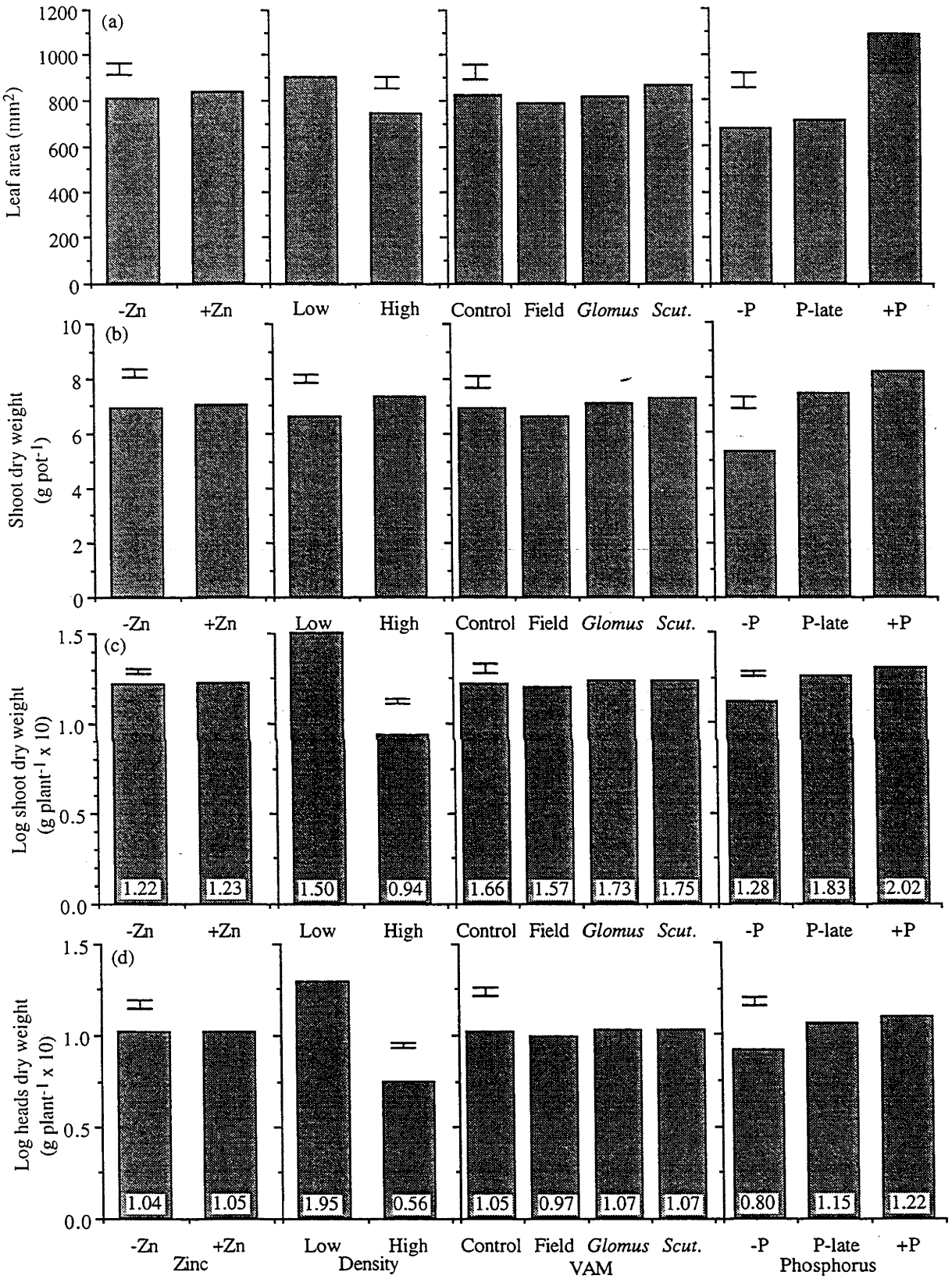


Figure 7.4. Estimated means and LSD at p=0.05 for a) area of the largest two leaves at week 3 (mm^2 pot^{-1}), b) shoot dry weight at harvest (g pot^{-1}), c) log shoot dry weight at harvest (g $plant^{-1}$) and d) log heads dry weight at harvest (g $plant^{-1}$). Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column. There were no significant interaction terms.

At harvest, shoot weight (g pot^{-1}) was examined to assess whether the density treatments had reached the same final yield. This was not the case, with shoot weight still being slightly lower at the low density (Fig. 7.4.b), thus all further analysis of harvest results was made at the level of individual plants.

Shoot dry weight ($plant^{-1}$, including heads) at harvest was not significantly affected by Zn (Fig. 7.4.c). Density had a large significant effect on shoot weight with plants in the low density pots being much heavier. The VAM treatment was close to being significant with the field treatment slightly lighter than the control and the *Glomus* and *Scutellospora* treatments slightly heavier than the control. When VAM was included as a continuous variable, VAM (%), it had a slight positive significant effect (Table 7.5). The same trend was evident when the biomass of roots colonised by VAM fungi was substituted for VAM (%) (results not shown). Addition of P markedly increased shoot growth, with P-late resulting in a similar shoot weight to when P was added before sowing. Similar trends were evident for the weight of the heads (grain plus glumes) (Fig. 7.4.d).

Results from ANOVAs of root dry weight and the root-shoot ratio are presented in Table 7.6 and Figure 7.5. Zinc had a slight positive effect on root weight. There was little difference between the non-VAM control and the *Glomus* and *Scutellospora* treatments, however, the field soil had a significantly lower root biomass. Phosphorus increased root biomass and the plants in the low density treatment had a greater root biomass. There was a significant interaction between P and density, with P-late being similar to +P at high density, but being intermediate to +P and -P at low density.

Table 7.6. Results from ANOVAs of root weight (g $plant^{-1}$) and the root-shoot ratio at harvest. Parameters included are block (1-4), Zn addition (-Zn, +Zn), density (low, high), VAM (control, field, *Glomus*, *Scutellospora*) and P addition (-P, P-late, +P). All significant interaction terms are included.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Log root dry weight	full model	83.1	<0.0001	0.86	158
	block	19.2	<0.0001		
	Zn	4.6	0.03		
	VAM	5.2	0.002		
	density	715.6	<0.0001		
	P	35.2	<0.0001		
	P x density	3.52	0.03		
Root-shoot ratio	full model	3.04	0.002	0.12	156
	block	0.8	0.5		
	Zn	1.2	0.3		
	VAM	1.9	0.1		
	density	1.9	0.13		
	P	11.2	0.001		

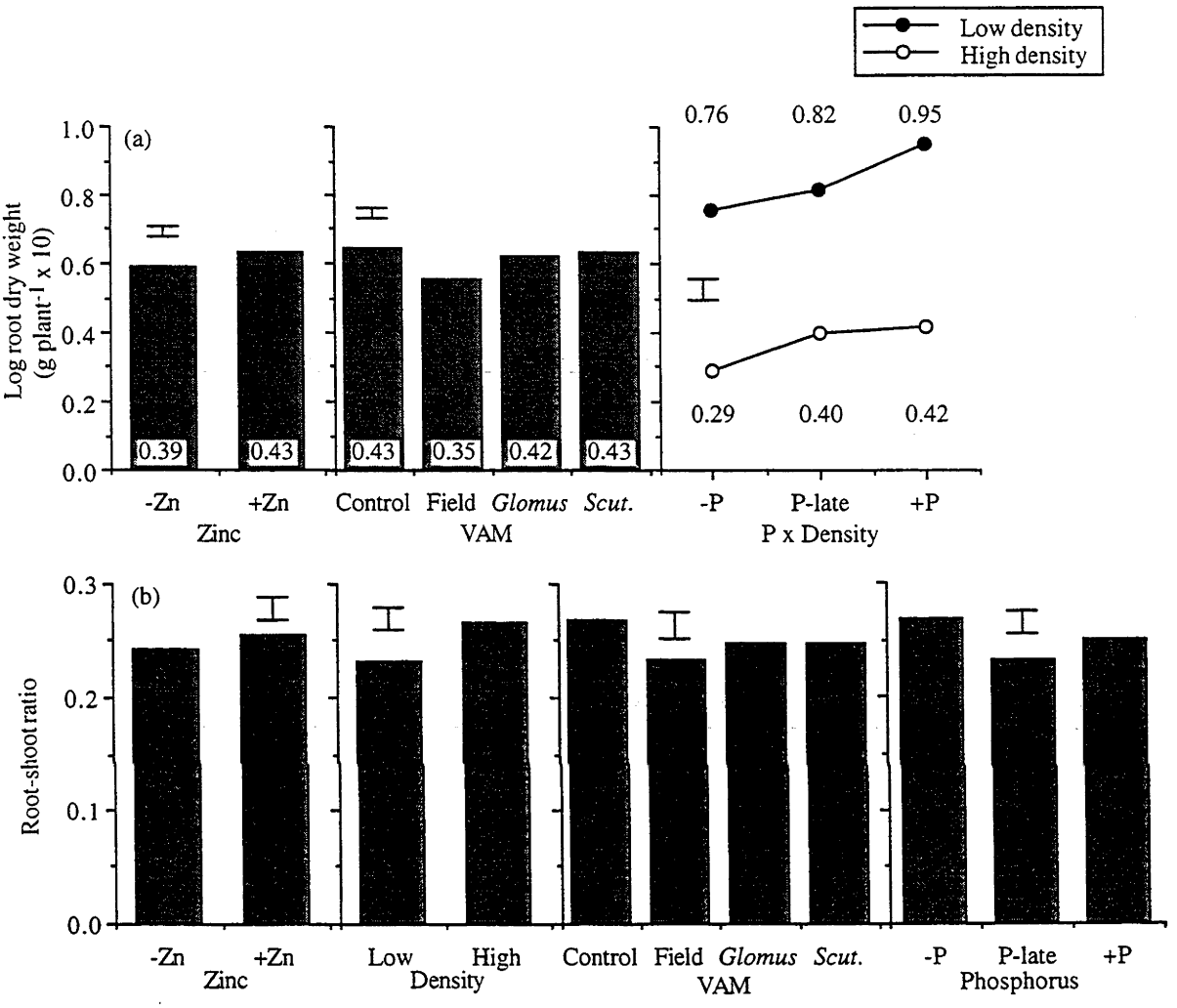


Figure 7.5. Estimated means and LSD at p=0.05 for a) root dry weight at harvest (g plant^{-1}) and b) the root-shoot ratio at harvest. Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column or close to the data point on the interaction graph. All significant interaction terms are shown.

The model for the root-shoot ratio did not explain as much variation as the other models and the adjusted r^2 was low (0.12). Zinc and VAM had no effect on the root-shoot ratio, although the field treatment tended to have a lower root-shoot ratio than the control. Plants at high density had a higher root-shoot ratio than plants at the lower density and addition of P decreased the root-shoot ratio (Fig. 7.5.b).

As addition of P either eliminated or markedly reduced VAM colonisation, two measures of plant growth were reanalysed with the P and P-late treatments excluded (Table 7.7). Results were similar to those in Tables 7.5 and 7.6, with VAM and Zn having no effect on shoot biomass and plants at low density being significantly larger than those at the higher density (Fig. 7.6.a). VAM inoculation tended to decrease root growth, while addition of Zn had a slight positive effect and plants at low density had a much larger root biomass than those at the higher density (Fig. 7.6.b). When VAM was included as a continuous variable it exerted a slight significant positive effect on shoot growth (Table 7.7).

Table 7.7. Results from ANOVAs and an ANCOVA of shoot dry weight (g plant^{-1}) and root dry weight (g plant^{-1}). Parameters included are block (1-4), Zn addition (-Zn, +Zn), density (low, high), and VAM (control, field, *Glomus*, *Scutellospora*). Treatments where P was added (+P and P-late) were excluded. Shoot weight (g plant^{-1}) was also modelled with the continuous measure, VAM %; the co-efficient for VAM (%) was 0.001 ± 0.0006 (se). There were no significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Log shoot dry weight	full model	55.0	<0.0001	0.87	63
	block	8.5	0.0001		
	Zn	0.4	0.5		
	VAM	2.4	0.08		
	density	404.3	<0.0001		
Log shoot dry weight	full model	72.0	<0.0001	0.87	63
	block	8.8	0.0001		
	Zn	0.3	0.6		
	VAM (%)	4.3	0.04		
	density	396.5	<0.0001		
Log root dry weight	full model	38.1	<0.0001	0.83	62
	block	6.5	0.0008		
	Zn	4.8	0.03		
	VAM	2.6	0.06		
	density	266.4	<0.0001		

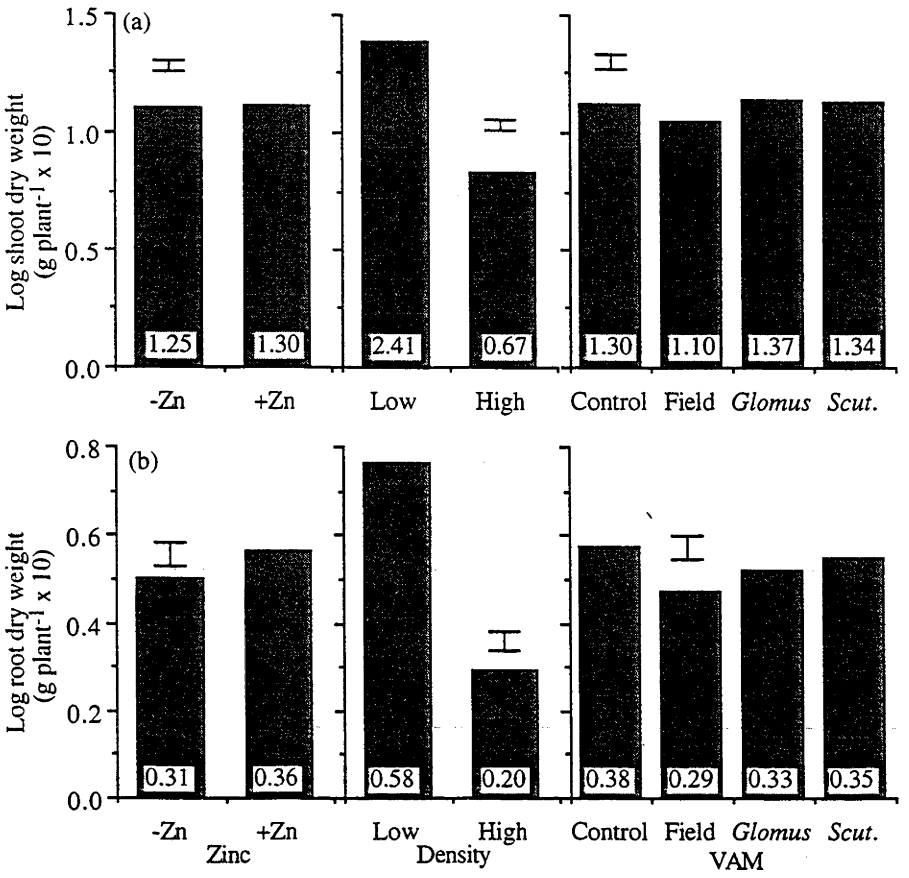


Figure 7.6. Estimated means and LSD at p=0.05 for a) log shoot dry weight at harvest (g plant^{-1}) and d) log root dry weight at harvest (g plant^{-1}). Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column. Treatments where P was added (P and P-late) were excluded. There were no significant interaction terms.

7.3.d. Discussion

(i) VAM Colonisation

The *Glomus* inoculum caused a much higher level of VAM colonisation than the *Scutellospora* inoculum. Such differences are expected between species of VAM fungi (Edathil *et al.* 1996) and in this instance, the lower internal colonisation in the *Scutellospora* treatment may have reflected the copious quantities of external vesicles and hyphae which were associated with colonisation by this fungus. The field soil inoculum resulted in very variable colonisation levels (0-20%) that were generally < 5%. This was unexpected and must have reflected inadequate amounts of inoculum being added. In addition, the soil used as inoculum was collected from under an organic wheat crop prior to anthesis and thus most inoculum in the soil may have been used by the crop, while production of spores may not have yet begun. Spores produced during the early stages of crop growth may have been dormant (Tommerup 1985). However, the inoculum did contain many roots from this organic crop, which should

have been highly colonised (Chapter 5).

Addition of Zn had no effect on VAM colonisation, a result consistent with the findings of Thompson (1990) who, in a similar glasshouse trial, applied the same concentration of Zn. Density also had no effect on VAM colonisation. VAM colonisation has been found to decrease at high plant densities (Bååth and Hayman 1984; §11.3), but, perhaps root densities were not high enough for this to occur. Root densities in the trial were approximately 19 cm cm^{-3}, which were higher than in the field (Fig. 5.8), indicating that root density was not a factor influencing VAM colonisation levels in the field (see also §12.1.b.ii and Table 12.2).

Addition of P significantly reduced VAM colonisation, having a greater effect on *Scutellospora* than *Glomus*. Other researchers have found VAM species vary in their susceptibility to P additions (Thomson *et al.* 1986). The reduction in colonisation was much greater than reported by Thompson (1990), even though the rate of P addition and soil extractable P (13.8 and 14 $\mu g\ g^{-1}$) were very similar. High P and low light may interact to markedly reduce VAM colonisation (Son and Smith 1988). However, as light levels were > 600 $\mu mol\ m^{-2}\ s^{-1}$ at noon, this seems unlikely to have occurred. The rate of P application, 50 mg kg^{-1} of soil, is approximately equivalent to 60 kg ha^{-1} (on an area basis). In fertiliser trials conducted at Ardlethan by Dann *et al.* (1996), 40 kg ha^{-1} of P caused a drop in VAM colonisation from 58% to 20%. In the glasshouse trial, the reduction in *Glomus* colonisation when P was applied, 55% to 25%, is consistent with these field results. Identification of the VAM species present on the Ardlethan farms was not attempted during this project. However, in a preliminary study by Ryan (1992), the morphology of internal colonisation was examined and it was concluded that the majority of colonisation was by *Glomus* spp., with some *Acaulospora* spp. also present. There was no evidence of *Scutellospora* being present.

The P-late treatment was intended to assess whether adding P after the plants and the fungi were established would allow a higher level of VAM colonisation to be maintained — see Aziz *et al.* (1991) — and the VAM fungi to therefore make a greater contribution to plant growth than in the +P treatment. However, the P-late treatment reduced VAM colonisation to a similar extent to the + P treatment, perhaps because VAM colonisation was not well established at week 2.

(ii) Plant Growth

Three measures of plant growth were assessed — area of two largest leaves at week 3, shoot dry weight and heads dry weight — as the contribution of VAM fungi to plant growth may vary with plant age and benefits may only become obvious at seed production (see Ronsheim *et al.* 1996). However, all three measures of shoot growth exhibited similar trends. In addition, it is useful to check for early evidence of differing

plant growth in the VAM treatment, as the fungi should not be influencing growth until they become established and seed reserves are exhausted (Allsopp and Stock 1995). Thus early large effects could be due to confounding factors — such as introduction of disease organisms in inoculum or non-random selection of seeds or seedlings (§7.2.d.iii) — and in glasshouse trials, initial small differences in plant growth may never be overcome (Black 1957; Derrick and Ryan 1998). However, the week 3 results (Fig. 7.4.a) do not show any unexpected effects.

Addition of Zn had no effect on shoot growth, but, increased root biomass by approximately 10%. The Zn treatment was included as Thompson (1990), on a soil from the northern wheatbelt, reported that VAM fungi increased Zn concentration in wheat. On the red earths used in this trial, Zn is generally not considered to limit wheat growth. Derrick (1996) found Zn concentrations in wheat crops at tillering in 1993 at Ardlethan to be generally lower in the conventional crop (16.2 mg kg^{-1}) than in the organic crop (24.2 mg kg^{-1}). Concentrations in the grain were 15.6 mg kg^{-1} in the conventional grain and 20.9 mg kg^{-1} in the organic grain which, as the concentrations were > 10 mg kg^{-1}, indicated that Zn nutrition was adequate in both crops (Derrick 1996). Thus the increase in root biomass in this trial was unexpected, but, overall there was no indication that Zn was a significant limiting factor for wheat growth or yield.

The plants in the low density pots reached a total combined shoot dry weight just below the shoot weight of the plants in the high density pots. Thus plants at the higher density were individually under a greater level of competitive stress than plants in the low density pots. The contributions of VAM fungi to plant growth have been found to vary with plant density, or the volume of soil available (Bååth and Hayman 1984), however there was no interaction between density and VAM in the present trial.

Addition of P increased shoot growth by 58%, indicating P was severely limiting plant growth. This is consistent with the results presented in Chapter 5 and those from fertiliser trials conducted at Ardlethan by Dann *et al.* (1996). Root biomass of individual plants was also increased by P; 45% at the high density and 25% at low density. Wheat root biomass will increase as P availability increases until P is extremely abundant; root growth is then suppressed (Riley *et al.* 1993; Tennant 1976). It is therefore curious that in the field results reported in Chapter 5, the organic crops, which were limited by P, had a greater root biomass than the conventional crops. Examining root-shoot ratios may be more useful in this instance.

The root-shoot ratio in the glasshouse trial was affected significantly by density and P. Plants at the lower density and plants which received P both had a lower root-shoot ratio, that is, contributed relatively less biomass to root growth. Riley *et al.* (1993) in a glasshouse trial examining wheat and P also found that increasing P decreased the root-shoot ratio. This is the expected response when supply of a limiting nutrient is increased (Wilson 1988b) and is consistent with the lower root-shoot ratios

found on the conventional farms described in Chapter 5. However, in the glasshouse trial, the effect of density was much greater than the effect of P. At high density, where competition between plants was greater, there was more allocation to root growth. This is also consistent with the field results where the highest root-shoot ratios were in the crops where weed levels and competitive stress — albeit interspecific, not intraspecific — were greatest (Table 5.11).

(iii) Effect of VAM Colonisation on Wheat Growth

Three inoculum treatments were used in the trial, as VAM species are known to differ in their effects on plant growth (Edathil *et al.* 1996; Gavito and Varela 1995) and the composition and functioning of VAM populations may adapt in response to fertilisation practices in agricultural systems (Johnson 1993; see §12.3.c.ii). Unfortunately, the field inoculum did not produce sufficient colonisation for comparisons to be made between the indigenous fungi and the other species. However, both the *Glomus* and *Scutellospora* fungi, which are from different genera, had an identical small effect on wheat growth indicating that factors other than the species of VAM fungi used were responsible for the small response of wheat to VAM inoculation in the trial.

The VAM treatment had a slight significant positive effect on total shoot growth, but not on the dry weight of the heads. Total shoot dry weight in each pot was around 5% greater in the *Glomus* and *Scutellospora* treatments than the non-VAM controls. VAM effects were slightly more significant, but still quite small, when VAM was included in the models as the continuous variable VAM (%). The field treatment, which had low and variable colonisation, generally resulted in lower plant growth than the control, which may indicate the introduction of pathogens (see also Thompson 1990 and §7.2.d.iii). The organic crop under which the soil used in the trial was collected contained areas of bare soil and stunted crop growth later in the season, strongly indicating the presence of a soil fungal pathogen, probably Rhizoctonia bare patch (*Rhizoctonia solani*) (Perry and Hillman 1991). However, there was no visual evidence of disease in the glasshouse trial and all treatments received filtrate from unsterilised field soil.

Thus, in spite of heavy VAM colonisation and P-limiting conditions, VAM fungi had a minor effect on wheat growth. It appeared that the benefits provided by VAM colonisation for wheat growth, presumably including enhanced P uptake, were being nearly completely offset by the use of photosynthate; 10-20% of photosynthate is required for maintaining VAM colonisation (Jakobsen and Rosendahl 1990; Peng *et al.* 1993). The high colonisation levels and the high intensity of the colonisation by the *Glomus* and *Scutellospora* suggests the fungi were using a substantial amount of photosynthate.

The strongly negative effect of VAM colonisation on plant growth observed in the first trial (§7.2.) did not occur in this trial, in spite of much higher VAM colonisation levels. This supports the hypothesis that low light levels were responsible for the parasitic effects observed in the first trial. Maximum daily light levels were 200-400 μmol m^{-2} s^{-1} during the first trial and 600-1400 μmol m^{-2} s^{-1} during the second trial. In addition, the first trial was conducted in early winter and the second in early summer when day lengths were longer; see Daft and El-Giahmi (1978).

However, the small positive effect of VAM fungi on wheat growth in the second trial (5%) does contrast with the findings of Thompson (1990) who, in a similar glasshouse trial on soil with an identical level of Colwell extractable P, found VAM colonisation increased grain yield by up to 37%. Although, this increase was not significant and when the data were analysed across all treatments, VAM inoculation significantly increased grain yield by 12.3%. The greater effect of VAM fungi on wheat growth found by Thompson (1900) could be due to differences in wheat cultivars (Kite vs Banks) (Baon *et al.* 1993b), environmental conditions (Manske 1989), VAM species or strains (Gavito and Varela 1995; Graham *et al.* 1982) or differences in other soil characteristics. Thompson (1990) also found VAM colonisation to have a large positive effect on shoot P and Zn concentrations. These were not measured in this trial, but, if VAM colonisation was increasing nutrient concentrations, it was not reflected in shoot growth or yield.

It is still possible that in the field there may be positive benefits from VAM colonisation for wheat growing in the soil used for these trials as, in a number of aspects, the glasshouse trial did not mimic field conditions. In particular, the higher temperatures in the glasshouse allowed more rapid plant growth and progression through the wheat lifecycle than would occur in the field. However, the results in this chapter are consistent with those found in the field (Chapter 5) and strongly suggest that any nutritional benefits from VAM colonisation for wheat grown in the soil used for these trials are, at best, minimal; even when plants are grown both in soil from an organic farm with low extractable P and without addition of soluble P fertiliser. Indeed, when Thompson (1987) compared growth of wheat in the field after short and long fallows in the northern Australian grain belt, the lower VAM colonisation in the wheat grown after a long fallow did not significantly reduce wheat shoot growth (Table 1.2). In contrast, the results from the small subterranean clover trial (Table 7.3), indicated that clover is probably heavily dependent on VAM fungi in these soils.

7.4. Conclusions from Glasshouse Trials using Mixed Farm Soil

• Both heat sterilisation and gamma irradiation eliminated VAM fungi from field soil.

• Wheat grown in irradiated soil had a greater biomass than wheat grown in heat sterilised soil or field soil.

• VAM colonisation levels were significantly reduced by addition of P.

• Plant density and Zn did not effect VAM colonisation levels.

• Wheat growth was strongly limited by P, but not by Zn.

• Inoculation with either *Glomus intraradices* or *Scutellospora calospora* increased wheat growth by, at most, 5%.

• Under low light conditions, VAM fungi appeared act as a parasitic on wheat.

• The effect of VAM fungi on wheat growth did not differ with P or Zn addition.

• Two factors which may differ between glasshouse trials and field conditions, plant density and VAM species, did not influence the effect of VAM fungi on wheat growth.

• Growth of subterranean clover was greatly increased — 60% for shoots and 34% for roots — by VAM colonisation, even though light levels were low.

• Use of field soil as inoculum means the possibility of introduction of pathogens must be considered.

Chapter Eight

Effect of Drought on Colonisation of Cereal Crops by VAM Fungi and Crop Growth

This chapter presents data on VAM colonisation levels and growth of crops during the severe 1994 drought — growing season rainfall < 50% of the long term average — and subsequent to the drought in 1995. Sampling during 1994 was originally intended to follow-on from the 1993 fieldwork (Chapter 5) and was to involve a closer examination of P cycling on conventional and alternative farms, however this was modified after it became evident that a severe drought was in process. The major findings from this chapter are published in Ryan and Ash (1996).

8.1. Aims

The aims of this chapter were to investigate the following questions.

- What effect will severe drought have on the level of VAM colonisation in crops?
- What effect will severe drought have on crop growth?
- Will a severe drought affect the VAM inoculum potential of soil and thereby influence the VAM colonisation and growth of crops the following season?
- Is there any evidence that VAM fungi alleviate crop water stress?

8.2. The Effect of a Severe Drought on VAM Colonisation and Crop Growth

8.2.a. Methods

Crops were sampled on the organic and conventional (III) farms at Ardlethan and the conventional and biodynamic farms at Cootamundra. A first year wheat crop was sampled on both farms at Ardlethan, along with a second year crop on the organic farm (organic 2nd; Plate 8.2); the conventional farm had no equivalent paddock. At Cootamundra no first year wheat was sown, therefore a first year triticale crop was sampled on each farm, as well as a first year barley crop on the biodynamic farm and a fourth year wheat on the conventional farm. The triticale crop on the conventional farm at Cootamundra was a grazing variety and was periodically grazed throughout the season. Further information on crop management is presented in Table 8.1. Note that the organic second year crop at Ardlethan and the conventional triticale crop at Cootamundra were sown respectively one and two months prior to the rest of the crops. Sowing of the other crops was delayed as the farmers waited for rain, but they were eventually sown 'dry'.

Crops were sampled on 13 June (Ardlethan organic first year crop only), 18 July, 11 August and 5 October. At each sampling, 10-20 plants were collected and assessed for shoot dry weight and VAM colonisation (§3.3 and §3.5). Plants collected in October from the three crops at Ardlethan were analysed for shoot P and N concentration (§3.3). While the youngest 2-3 fully-emerged leaves were analysed from plants in the organic first year crop, whole plants were analysed from the other two crops, due to the small size of the plants. Crop yield figures were supplied by the farmers.

Plate 8.1. An aerial view of drought-affected wheat and canola crops in November 1994 showing the effects of tree lines and individual trees on crop growth (courtesy of Julian Ash).

Plate 8.2. A drought-affected second year wheat crop at Ardlethan in November 1994.

Table 8.1. Management details of cereal crops sampled in 1994. Active ingredients of chemicals are presented in Appendix 2.

Location	Farm management strategy	History since last pasture phase (years)	Crop	Pre-sowing	Sowing Date	Fertiliser (kg ha^{-1})	Nutrients applied (kg ha^{-1}) P	Others	Other
Ardlethan	conventional	3 pasture	wheat cv. Janz	Roundup 1 L ha^{-1} in October, 3 cultivations, first in December	13 June	superphosphate 62	14	7 N 1.4 S	No harvest; crop grazed
	organic	5 pasture	wheat cv. Banks (Organic 1st)	3 cultivations, first in October	13 May	Moroccan reactive phosphate rock 125.3	16	44 Ca 1.6 S	
		3 pasture, 1 wheat	wheat cv. Banks (Organic 2nd)	3 cultivations	13 June	-	-	-	No harvest; crop grazed Sampled in 1993
Cootamundra	conventional	5 pasture	triticale cv. Maiden	Roundup 1 L ha^{-1} in October, 2 cultivations, first in November	15 April	diammonium phosphate 80	18	16 N 1.4 S	Post-sowing; Tristar 1.5 2,4-D Ester 800 0.7 A grazing variety of triticale
		4 pasture, 1 oats, 1 wheat, 1 canola	wheat cv. Janz	Roundup 1 L ha^{-1} in October, 2 cultivations, first in November	28 June	diammonium phosphate 100	20	18 N 1.5 S	-
	biodynamic	5 pasture	triticale cv. Tahara	3 cultivations, first in October	14 June	Quinphos reactive phosphate rock 78.4	10	30 Ca 1.2 S	Undersown with clover No harvest; crop grazed
		3 pasture	barley cv. Malibu	summer forage sorghum, 2 cultivations	20 June	Quinphos reactive phosphate rock 78.4	10	30 Ca 1.2 S	Undersown with clover No harvest; crop grazed

On 18 July, soil was collected from 10 sites in each crop at Ardlethan and the triticale crops at Cootamundra. Soil pH was measured as described in section 3.6 and soil was sent to Wesfarmers CSBP (Perth, Western Australia) for analysis of extractable P (Colwell 1963).

Throughout the cropping season it was noted that there were large areas of bare ground and stunted crop growth around trees growing in, or adjacent to, crops (Plate 8.1). On 7 November wheat plants in the first year paddock at Ardlethan were sampled from around such an area. The trees, predominantly *Eucalyptus melliodora* A Cunn. ex Schau., were growing in a 3 m wide road reserve along an east-west fenceline on the southern side of the paddock and were approximately 17 m tall and had a trunk diameter of 0.45 m at the base. Adjacent to the trees for 20 m into the paddock the ground was bare with no growth of wheat or weeds and for the next 20 m, crop growth was stunted in comparison to the rest of the paddock.

Wheat plants were sampled from 15 transects, which ran for 20 m starting where the first wheat plants appeared and ended where crop growth no longer appeared stunted. The height of wheat plants increased consistently along each transect, as distance from the trees increased. One plant around the heights of 0.1, 0.2, 0.3, 0.4 and 0.5 m were collected in succession along each transect. Plant height was assumed to reflect the level of water stress the plant had experienced during the growing season. Shoot dry weight and VAM colonisation levels were assessed (§3.3 and §3.5). Crop development stage was estimated using the Zadoks scale (Zadoks *et al.* 1974). Monthly rainfall figures for Ardlethan and Cootamundra were supplied by the Bureau of Meteorology.

8.2.b. Results

(i) Rainfall

Table 8.2. Rainfall at Ardlethan and Cootamundra in 1994 (mm); monthly totals, total for the year and total for June to October, the portion of the growing season during which sampling occurred.

	J	F	M	A	M	J	J	A	S	O	N	D	Year	J-O
Ardlethan	2	140	58	4	1	20	11	15	4	9	32	11	308	61
Cootamundra	9	141	26	22	6	45	27	12	19	44	42	27	420	147

Rainfall at Ardlethan and Cootamundra in 1994 is presented in Table 8.2. Total rainfall at Ardlethan was 308 mm and rainfall between May and October was 61 mm. May to October rainfall was only 30% of the long term average for Ardlethan, making 1994 the worst drought on record. At Cootamundra the total and May to October rainfall were 420 and 147 mm respectively. May to October rainfall was approximately 50% of the long term average.

(ii) Soil Nutrient Concentrations

At Ardlethan, soil extractable P was much higher on the conventional farm than on the organic farm, while at Cootamundra the concentration was similar on the two farms. Soil pH was similar on all farms (Table 8.3).

Table 8.3. Colwell soil extractable P and pH in soil from conventional (Con.) and organic wheat paddocks at Ardlethan and conventional (Con.) and biodynamic (BD) triticale paddocks at Cootamundra in July 1994; mean *(s.e.m.)*, n=10.

	Ardlethan			Cootamundra	
	Con.	Organic 1st	Organic 2nd	Con.	BD
Extractable P ($\mu g\ g^{-1}$)	43.3 *(5.3)*	9.7 *(0.4)*	9.7 *(0.4)*	39.9 *(3.3)*	36.7 *(4.6)*
pH	5.6	5.8	5.8	5.8	5.9

(iii) VAM Colonisation of Crops

VAM colonisation of the crops at Ardlethan over the 1994 season is shown in Figure 8.1.a. On the conventional farm and in the second year crop on the organic farm VAM (%) was extremely low. In the first year paddock on the organic farm, colonisation increased steadily over the season, with around 40% of root length eventually becoming colonised. VAM colonisation in crops at Cootamundra is shown in Figures 8.1.b and 8.1.c. VAM colonisation was low in all crops, ranging from 3% of root length in the conventional triticale to 22% in the biodynamic barley.

(iv) Crop Growth, Nutrition and Yield

At Ardlethan, shoot dry weight was extremely low in the conventional crop and the second year organic crop, but was slightly higher in the first year organic crop (Fig. 8.2.a). Both the conventional crop and the second year organic crop were too poor to harvest and were left for stock to graze, while the first year organic crop was harvested, yielding 2 t ha^{-1}.

At Cootamundra, shoot dry weight was also low on all farms, with the earlier sown — and periodically grazed — conventional triticale reaching approximately twice the weight of the other crops (Figs. 8.2.b and 8.2.c). Crops on the biodynamic farm were too poor to harvest and were grazed by stock, while on the conventional farm the wheat yielded 1.8 t ha^{-1} and the triticale 2 t ha^{-1}.

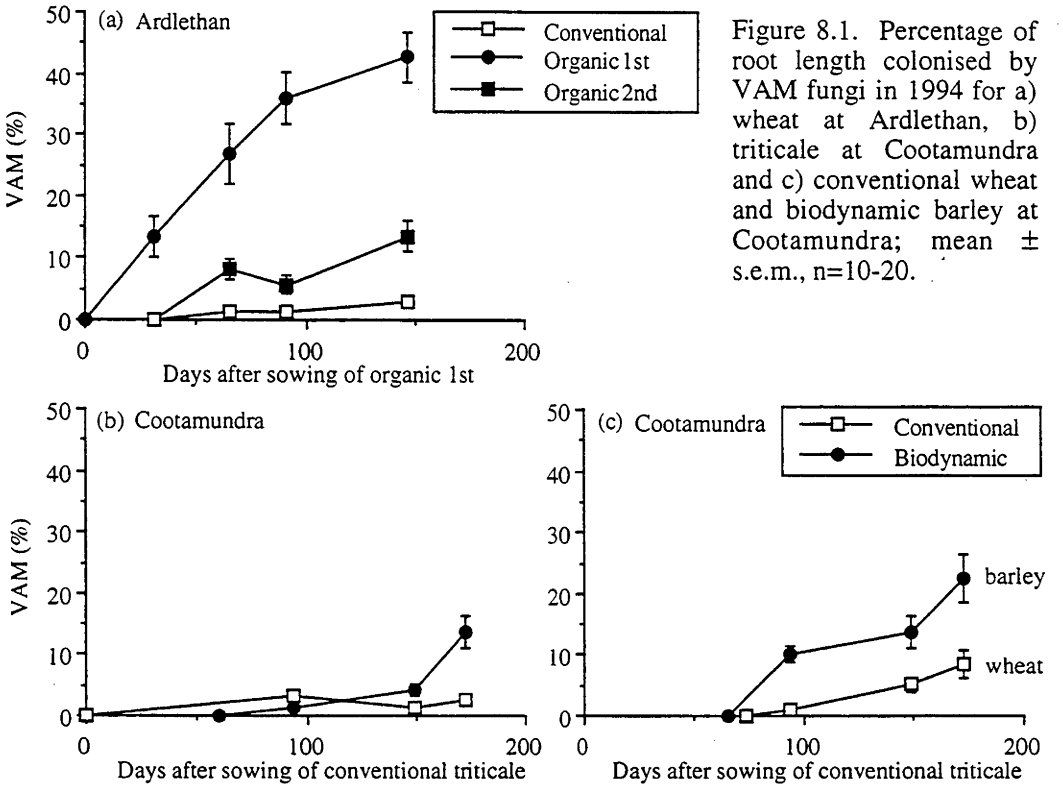


Figure 8.1. Percentage of root length colonised by VAM fungi in 1994 for a) wheat at Ardlethan, b) triticale at Cootamundra and c) conventional wheat and biodynamic barley at Cootamundra; mean ± s.e.m., n=10-20.

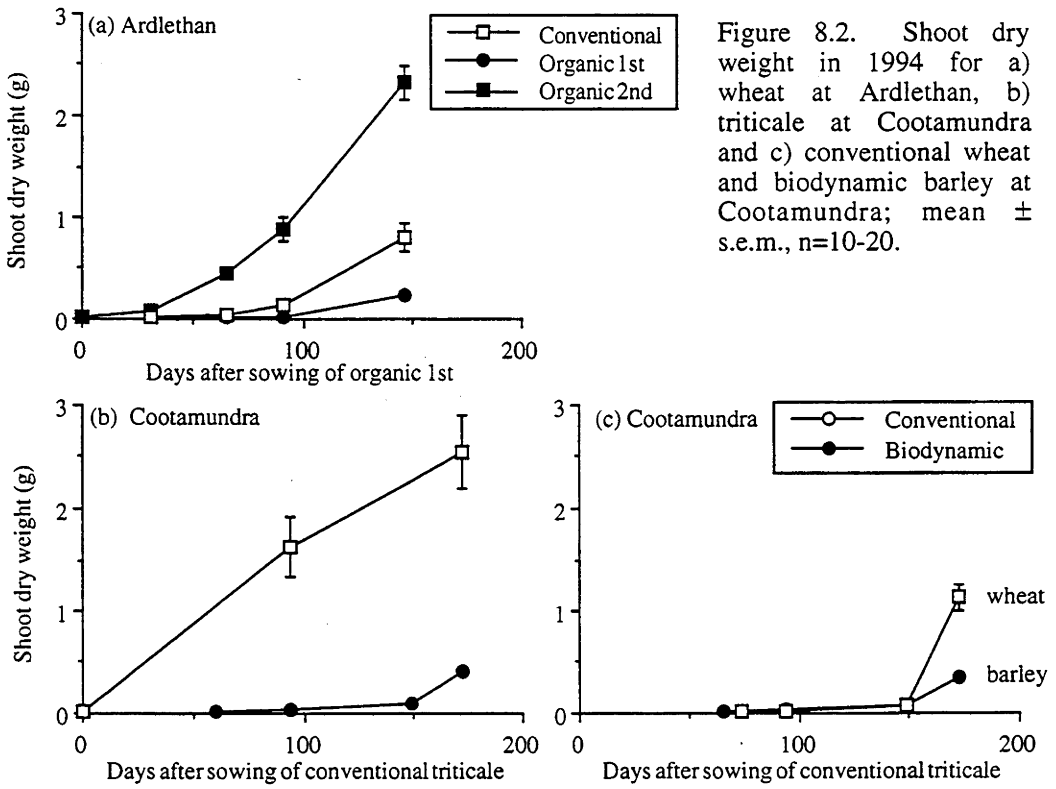


Figure 8.2. Shoot dry weight in 1994 for a) wheat at Ardlethan, b) triticale at Cootamundra and c) conventional wheat and biodynamic barley at Cootamundra; mean ± s.e.m., n=10-20.

Shoot P and N in the Ardlethan crops are presented in Table 8.4. Although whole plants were not analysed in one of the paddocks, it is unlikely that the values differed much from whole plant values at this growth stage; the small difference in development stage is also unlikely to have had a large influence on the results. Nutrient concentrations were similar for the two organic crops. Shoot P in the conventional crop was around three times higher than the other crops and shoot N was also slightly higher in the conventional crop.

Table 8.4. Shoot P and N in crops at Ardlethan on 3 October; mean *(s.e.m.)*, n=10.

	Conventional	Organic 1st	Organic 2nd
Sample	whole plant	2-3 leaves	whole plant
Growth stage	booting	anthesis	booting
P (%)	0.45 *(0.02)*	0.15 *(0.005)*	0.17 *(0.005)*
N (%)	4.4 *(0.05)*	3.4 *(0.05)*	3.5 *(0.14)*

(v) VAM Colonisation and Crop Growth around Trees

Throughout the 1994 cropping season there were large areas of bare ground around trees (Plate 8.1). Figure 8.3 presents the VAM (%) of wheat plants collected from a section of the Ardlethan organic first year wheat paddock which was adjacent to trees. Height of plants increased as distance from the trees increased and colonisation by VAM fungi also increased, from 11 to 32% of root length. Plants of all heights were forming heads, however root system development was less advanced on the plants closest to the trees, where there was no development of nodal roots (Table 8.5). Plants closest to the trees resembled the more severely drought affected plants in the second year organic crop (Plate 8.2) and conventional crop at Ardlethan.

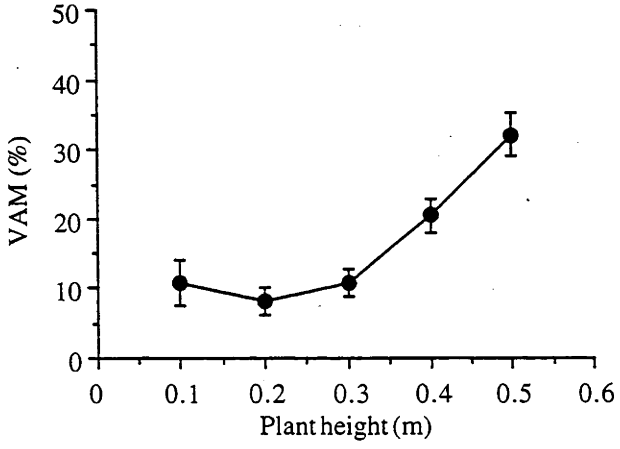


Figure 8.3. Percentage of root length colonised by VAM fungi in wheat plants adjacent to trees in the organic first year paddock in November 1994; mean ± s.e.m., n=15. Plant height increased with distance from trees.

Table 8.5. Nodal roots, development stage (Zadoks scale) and number of tillers of wheat plants adjacent to trees in the organic first year wheat paddock on 7 November; plant height increased with distance from trees.

Plant height (m)	Nodal roots	Development stage	Tillers
0.1	no	Z49-59 (ear emergence)	0
0.2	no	Z61-69 (flowering)	0
0.3	short	> Z70 (milk development)	0
0.4	1 or 2 long roots	> Z70	0
0.5	many, well developed	> Z70	0-1

8.2.c. Discussion

(i) VAM Colonisation Levels

VAM colonisation levels varied with both plant growth and farm management strategy. On the conventional farms, final VAM colonisation ranged from 2-8%, while on the alternative farms colonisation ranged from 13-40%. This was much lower than 1993 at anthesis when colonisation in first year wheat ranged from 28-36% on conventional farms and 55-71% on alternative farms (Fig. 5.1). The rate of colonisation also differed from 1993 with the initial rate of colonisation being much slower in 1994. These results strongly suggest drought conditions as a major influence on VAM colonisation in 1994.

In the first year wheat crop at Ardlethan, crop growth greater and VAM colonisation levels were higher than in the other two crops. However, in areas of this paddock which were adjacent to trees, VAM colonisation was reduced to levels similar to those in the other Ardlethan crops, presumably due to the trees competing with the crop for water. Allelopathic interactions and shading — which could have been contributing to the reduced VAM colonisation and growth of these plants — were unlikely to have been significant, as eucalypt litter in the crop was negligible and the reduced VAM colonisation was found on unshaded plants up to 40 m north of the trees, which only had a canopy radius of 4-6 m and a height of 8-17 m. Moreover, when an individual tree was surrounded by crop, the effect on crop growth occurred in a circular pattern well beyond the canopy (J. Ash, unpublished data; Plate 8.1), which is not consistent with microclimate shading being the primary cause.

Both the conventional triticale crop at Cootamundra and the organic first wheat at Ardlethan reached a greater shoot dry weight than the other crops and therefore were presumably were less affected by the drought (§8.2.c.ii). The high VAM colonisation levels in the wheat (40%) may have resulted from a high level of colonisation being reached before drought conditions began to affect growth of both the crop and the fungi. However, the triticale was poorly colonised (3%). The reason for this negligible colonisation is unclear as soil extractable P was similar to the neighbouring biodynamic farm, where VAM colonisation reached 13% in triticale (Fig. 8.1 and Table 8.3). The

relatively high dry weight of the conventional triticale makes it unlikely that the low VAM levels were due to P accumulation in the plants (shoot P was not measured). The potential for triticale to be highly colonised by VAM fungi is shown by the 1995 biodynamic triticale crop where 41% of root length was colonised (Fig. 8.4). It is possible that colonisation in the conventional triticale reached a peak before the first sampling at 95 days after sowing and then declined, or perhaps was inhibited by the periodic grazing.

Overall, it seems clear that the low levels of VAM colonisation in most of the crops sampled in 1994 were due to the drought (see §12.1.a.iii). In a glasshouse trial, Mohammad *et al.* (1995) found that water stress — watering pots to field capacity every three days as opposed to every day — halved VAM colonisation in wheat. Accumulation of P in the plants due to the drought may also have affected VAM colonisation, particularly in the Ardlethan conventional crop. However, the poorly colonised second year crop on the organic farm had a shoot P concentration of 0.17%, only slightly higher than the 1993 crops (0.14%).

In a large number of both field and glasshouse experiments — reviewed by Sánchez-Díaz (1994) — there are indications that VAM fungi may improve the tolerance of host plants to water stress by VAM fungi either enhancing nutrient uptake under drought conditions (Puppi and Bras 1989; Sylvia *et al.* 1993) or directly influencing plant water relations (Auge *et al.* 1986; Bethlenfalvay *et al.* 1988). In other instances, VAM fungi have been found to have no effect on the drought tolerance of the host plant (Daniels Hetrick *et al.* 1984; Fitter 1988; Simpson and Daft 1990). Overall, the topic of VAM and plant water relations is complex and there are many inconsistencies in the literature (Smith and Read 1997). Smith and Read (1997) conclude that most, if not all, effects of VAM fungi on plant water relations are likely to be a secondary effect of changes in plant nutrient status caused by the VAM fungi. In the severely drought-affected 1994 crops, the negligible VAM colonisation makes it unlikely that the fungi were making any significant contribution towards alleviating crop water stress.

(ii) Crop Growth

Crop growth in 1994 was very poor at both Ardlethan and Cootamundra. Large areas of bare ground and retarded crop growth around trees indicated that water was limiting crop growth; this effect was not as prominent in 1993 or later years (Plate 8.1, J. Ash, unpublished data). Shoot dry weights at anthesis in the conventional first year crop at Ardlethan were 0.8 g in 1994 and 17.4 g in 1993 and on the organic farm, 2.3 g in 1994 and 7.4 g in 1993.

The two crops which reached the greatest dry weight and produced a harvestable crop, although not a high yield, were the conventional triticale at

Cootamundra and the first year organic wheat at Ardlethan. The triticale crop was sown two months earlier than any of the other crops at Cootamundra and presumably was able better utilise the soil moisture remaining from the 141 mm of rain which fell in February. The organic first year crop at Ardlethan, as well as being sown on 13 May — a month before the other crops which were sampled — also had two months extra fallow after the first cultivation in October, which would have resulted in greater stored soil moisture (Fettell 1980). In early May, soil in this paddock below 50 mm depth was visibly damp, while in the other two paddocks at Ardlethan there were no visible signs of moisture. All other crops at Ardlethan and Cootamundra were sown 'dry' in anticipation of adequate rainfall, which did not occur.

The conventional and second year organic crops at Ardlethan and the wheat plants closest to the trees in the first year crop had retarded root system development (Table 8.5), with no growth of the nodal roots which normally appear around four weeks after sowing (Tennant 1976). This may have been the result of severe early water stress causing the plants to allocate all resources into producing at least some viable seed, instead of concentrating resources on root growth, as has been noted elsewhere under dry conditions of lesser severity (Hamblin *et al.* 1990).

Shoot P and N concentrations in the Ardlethan crops also indicated water was limiting growth (Table 8.4). Wheat shoot P > 0.12% at anthesis can be considered to be normal (Reuter and Robinson 1986) and thus the organic crops contained adequate P (0.15%, 0.17%). The shoot P of 0.46% in the conventional crop can be considered to be high, but not toxic (Reuter and Robinson 1986). In 1993, both first year crops sampled at Ardlethan contained 0.14% P in shoots at anthesis. As wheat shoot N > 1.4% at anthesis is normal (Reuter and Robinson 1986), the organic crops had a higher than adequate concentration of N (3.4%, 3.5%). The higher shoot N in the conventional crop (4.4%) was perhaps due to the application of 62 kg ha^{-1} of superphosphate. In 1993 at anthesis, shoot N was 1.3% and 1.4% in the conventional and organic crops respectively. The higher concentrations of nutrients in the 1994 crops, especially the conventional crop which was the only one to receive soluble fertilisers, strongly indicates that nutrients were accumulating in excess of the needs of the plants, due to growth being limited by lack of water (Verasan and Phillips 1978).

8.3. VAM Colonisation and Crop Growth Post-Drought

In 1995, crops at Ardlethan and Cootamundra were sampled to assess whether the low VAM colonisation during the 1994 drought would carry-over and effect VAM colonisation levels and crop growth. Additional information on 1995 crops from a preliminary study on soil structure and VAM hyphal length under the crops at Ardlethan in 1995 is contained in Gatehouse (1995).

8.3.a. Methods

Crops were sampled on the organic and conventional (III) farms at Ardlethan and the biodynamic and conventional farms at Cootamundra. There were no first year crops available for sampling making it difficult to consistently match paddocks by rotation stage, however all crops sampled in 1995 were in paddocks which had contained a severely drought-affected crop in 1994 (Table 8.6). Wheat crops were sampled on each of the four farms and a triticale crop was also sampled on the biodynamic farm. Additional information on crop management is presented in Table 8.6.

Paddocks were sampled on 21 June (Ardlethan only), 7 July (Cootamundra only), 12 August and 13 October. Soil was collected from 10 sites in each paddock on the first sampling date and sent to Wesfarmers CSBP (Perth, Western Australia) for analysis of extractable P (Colwell 1963). Soil pH was measured as described in section 3.6. VAM colonisation levels and shoot dry weights were determined (§3.3 and §3.5). Crop yield figures were supplied by the farmers and weed levels estimated visually. Rainfall figures were supplied by the Bureau of Meteorology with missing data points for Ardlethan extrapolated from neighbouring stations.

8.3.b. Results

Rainfall figures for 1995 are presented in Table 8.7. Annual and growing season (June to October) long term averages are 487 and 211 mm at Ardlethan and 625 and 388 mm at Cootamundra, thus annual rainfall was above average, while growing season rainfall was slightly below average. Rainfall was low in February and March, but there had been substantial falls by the time crops were sown in late May.

Table 8.7. Rainfall at Ardlethan and Cootamundra in 1995; monthly totals, total for the year and total for June to October, the portion of the growing season during which sampling occurred.

	J	F	M	A	M	J	J	A	S	O	N	D	Year	J-O
Ardlethan	130	4	0	18	96	60	50	4	24	28	60	38	512	166
Cootamundra	146	4	0	29	134	70	99	11	49	71	163	48	825	300

Table 8.6. Management details of cereal crops sampled in 1995. Active ingredients of chemicals are presented in Appendix 2.

Location	Farm management strategy	History since last pasture phase (years)	Crop	Pre-sowing	Sown	Fertiliser (kg ha^{-1})	Nutrients applied (kg ha^{-1}) P	Others	Post sowing (L ha^{-1})	Other
Ardlethan	conventional	3 pasture, 1 wheat (failed due to drought)	wheat cv. Janz	2 cultivations	30 May	superphosphate 62	14	6.0 N 1.4 S	-	Sampled in 1994
	organic	3 pasture, 1 wheat, 1 wheat (failed due to drought)	wheat cv. Banks	1 cultivation	23 May	Moroccan reactive phosphate rock 125.3	16.4	44.0 Ca 1.6 S	-	Sampled in 1993 and 1994
Cootamundra	conventional	4 pasture, 1 oats, 1 wheat, 1 canola, 1 wheat (failed due to drought)	wheat cv. Swift	2 cultivations	1 June	diammonium phosphate 110	22	19.8 N 1.7 S	Tigrex 1 Tristar 1.5 2,4-D Ester 800 0.7	Sampled in 1994
	biodynamic	3 pasture, 1 barley (failed due to drought)	wheat cv. Dollarbird	2 cultivations	24 May	Quinphos reactive phosphate rock 44.8 lime 203	10	25.9 Ca 1.2 S 80 Ca	-	Undersown with strawberry and white clover, phalaris, cocksfoot, Sampled in 1994
		3 pasture, 1 barley (failed due to drought)	triticale cv. Tahara	2 cultivations	9 May	Quinphos reactive phosphate rock 89.6 lime 203	20	51.8 Ca 2.4 S 160 Ca	-	Undersown with strawberry and white clover, phalaris, cocksfoot, Sampled in 1994

Soil extractable P at seedling stage (Table 8.8) was greater at Cootamundra than Ardlethan and was higher on the conventionally managed farms. Soil pH was similar in all paddocks.

Table 8.8. Colwell soil extractable P and pH in 1995 on the conventional (Con.)/organic farm pair at Ardlethan and the conventional (Con.)/biodynamic farm pair at Cootamundra; mean *(s.e.m.)*, n=10.

	Ardlethan		Cootamundra		
	Con. wheat	Organic wheat	Con. wheat	Biodynamic wheat	triticale
Extractable P ($\mu g\ g^{-1}$)	33.1 *(3.1)*	12.6 *(0.7)*	80.7*(2.9)*	37.6 *(0.5)*	61.2 *(1.8)*
pH	6.1	6.2	5.8	5.9	6.2

VAM colonisation curves for crops in 1995 are presented in Figure 8.4. At both locations, VAM colonisation in the conventional crops was lower than in the alternatively managed crops. At Ardlethan, VAM colonisation at anthesis was at a similar level to 1993; much higher than in 1994. At Cootamundra, VAM colonisation was also much higher than in 1994, although VAM (%) in the biodynamic wheat crop at anthesis was around 30% lower than in 1993. In all crops, the initial rate of VAM colonisation was slower than in 1993 (Fig. 5.1).

Figure 8.5 presents the change in individual shoot dry weights over the season. At Ardlethan, shoot dry weight was much greater on the conventional farm, while at Cootamundra the biodynamic crops reached a greater shoot dry weight than the conventional crops. In all cases, the initial growth of the crops appeared slower than in 1993 (Fig. 5.6). Crop yields mirrored the dry weight results, except the biodynamic wheat yielded less than the conventional wheat at Cootamundra (Table 8.9). Weed levels were very high in both the biodynamic wheat at Cootamundra and the organic wheat at Ardlethan (Table 8.9).

Table 8.9. Crop yield and weed levels in paddocks sampled in 1995.

	Ardlethan		Cootamundra		
	Conventional wheat	Organic wheat	Conventional wheat	Biodynamic wheat	triticale
Grain yield ($t\ ha^{-1}$)	3.2	1.1	3.8	0.7	5.1
Weed levels	negligible	high	medium	high	low

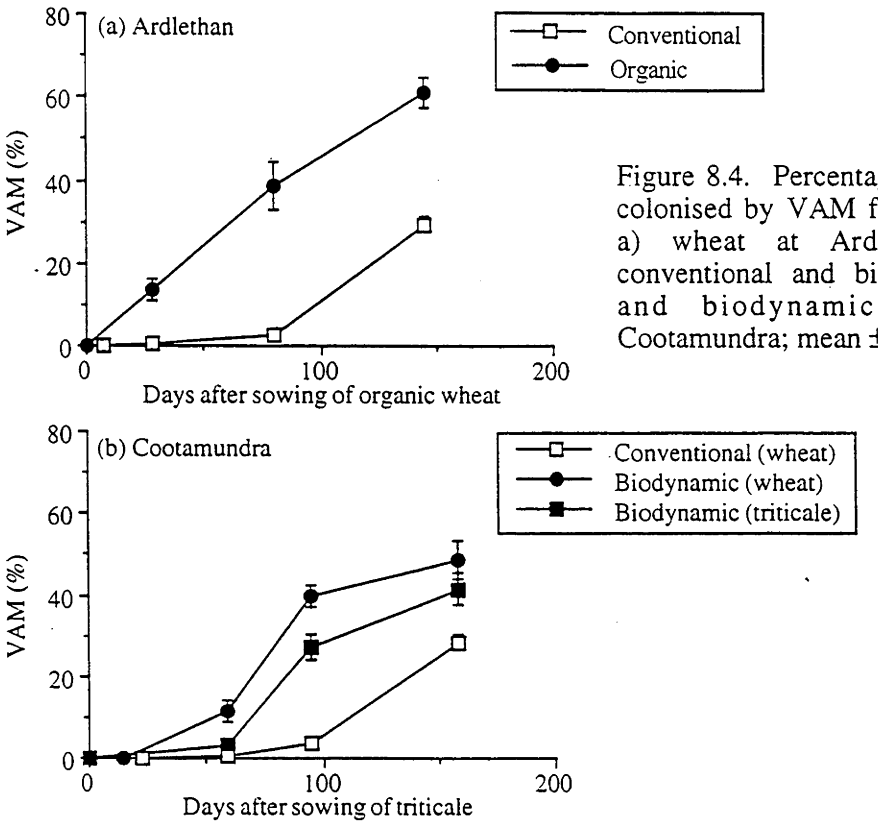


Figure 8.4. Percentage of root length colonised by VAM fungi in 1995 for a) wheat at Ardlethan and b) conventional and biodynamic wheat and biodynamic triticale at Cootamundra; mean ± s.e.m., n=10.

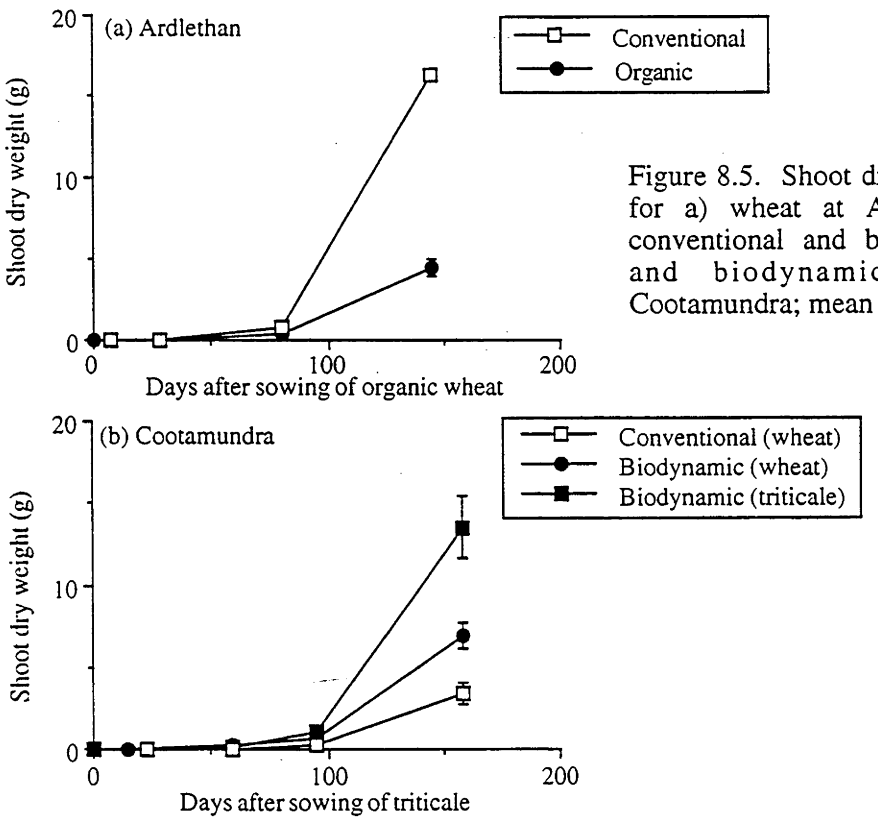


Figure 8.5. Shoot dry weight in 1995 for a) wheat at Ardlethan and b) conventional and biodynamic wheat and biodynamic triticale at Cootamundra; mean ± s.e.m., n=10.

8.3.c. Discussion

(i) VAM Colonisation

The primary aim of the 1995 fieldwork was to examine whether the 1994 drought had resulted in reduced levels of VAM colonisation in the 1995 crops. Using the curves of VAM colonisation over the 1992 (Ryan 1992), 1993 (Fig. 5.1), 1994 (Fig. 8.1) and 1995 (Fig. 8.4) seasons, values for VAM colonisation levels 50 and 100 days after sowing were estimated (Table 8.10). The negative effect of the 1994 drought on VAM colonisation is apparent on both conventional and alternative farms. On the alternative farms the post-drought 1995 crops had a markedly lower rate of colonisation than in 1992 and 1993. For instance, at Ardlethan in 1995, VAM (%) 50 days after sowing was half the levels in 1992 and 1993, and after 100 days it was less than two-thirds the colonisation level in these previous years. This lag in colonisation in 1995 strongly suggests that the drought had reduced inoculum in the soil below the levels usually present at the beginning of a wheat season.

Table 8.10. Percentage of root length colonised by VAM fungi in crops 50 and 100 days after sowing in 1992 (Ryan 1992), 1993, 1994 and 1995. All figures are for wheat crops except the 1994 biodynamic crop at Cootamundra which was barley. The 1994 figures for the organic farm at Ardlethan are from the badly drought-affected second year crop grown in the paddock from which the 1995 crop was sampled.

	Conventional		Alternative	
Days after sowing	50	100	50	100
Ardlethan				
1992	3	9	42	62
1993	8	33	48	68
1994	**1**	**2**	**3**	**10**
1995	2	10	22	44
Cootamundra				
1993	-	-	46	63
1994	**3**	**8**	**12**	**20**
1995	1	3	8	42

While the drought conditions — and resulting poor crop growth and low levels of VAM colonisation — during 1994 provide the most likely explanation for these results, it is possible that paddock history may also have had an influence. In 1992 and 1993 all crops sampled were the first crops sown after a pasture phase, although the biodynamic crop had been preceded by summer forage sorghum. In 1995, owing to the failure of crops in the 1994 drought, paddocks were being sown for the second or third

time which may have caused a reduction in VAM inoculum levels. It is also possible that high levels of competition from weeds may have reduced the rate of VAM colonisation in the 1995 organic crop at Ardlethan. However, this is unlikely, as crops on the biodynamic farm are routinely heavily undersown with a variety of pasture grasses and legumes (Plate 4.2) and in 1993 — although 30% of biomass consisted of species other than wheat — the initial colonisation rate was virtually identical to that on the organic farm at Ardlethan which had a negligible levels of weeds (Fig. 5.7).

VAM levels in the organic crop at Ardlethan in 1995 eventually reached 61%, a level similar to 1993. However, at Cootamundra, VAM colonisation reached only 49% of root length in the wheat crop and 40% in the triticale crop; much lower than the 69% reached in wheat in 1993. As extractable soil P at seedling was 19 $\mu g\ g^{-1}$ in 1993 (Derrick 1996) and 38 $\mu g\ g^{-1}$ in the wheat and 61 $\mu g\ g^{-1}$ in the triticale in 1995, it is possible that higher soil extractable P in 1995 was restricting colonisation.

The biodynamic triticale crop was included for sampling in 1995 to ascertain whether triticale can become highly colonised by VAM fungi and to ensure that the low colonisation levels in 1994, 4% and 14% (Fig. 8.1), were due to the drought. As colonisation levels reached 38% in 1995, it appears the 1994 levels were unusually low.

(ii) Crop Growth

At Ardlethan, the conventional crop had a much higher shoot dry weight than the organic crop and crop yield was also much greater; 3.2 t ha^{-1} compared with 1.1 t ha^{-1}. While the lower soil extractable P on the organic farm would have been responsible for some of this effect (Chapter 5), the organic crop also had a high level of weed infestation, similar to the organic crop at Yenda in 1993 (Table 5.4). Although no quantitative measure of weed biomass was made, it is obvious that crop growth and yield were significantly reduced by the weeds. The biodynamic wheat at Cootamundra was likely to have been similarly affected and while the biodynamic wheat had a greater shoot dry weight than the conventional, it yielded considerably less. This was probably largely a consequence of the variety sown (J. Orgill, pers. comm.).

Assuming that the slower rates of VAM colonisation were due to a reduction in inoculum due to the 1994 drought, it seems that a severe drought is — to some degree — equivalent to a long fallow. In the northern wheatbelt, long fallows were found by Thompson (1987) to reduce VAM colonisation levels in subsequent crops, thereby causing significant reductions in growth of some crops (§1.4); a phenomenon also reported in North America (Wetterauer and Killorn 1996). The 1995 crops initially had a slower growth rate than the 1993 crops, however, insufficient data were collected to repeat the modelling presented in Chapter 5 and draw solid conclusions about the causes of this difference. It seems unlikely to be due to rainfall, as although rainfall was low in February and March, substantial falls had occurred by the time the crops

were sown in late May. Alternatively, lower soil N in 1995 may have been, at least partly, responsible. The 1993 crops were all first year crops and had access to the biologically fixed N stored in the soil during the pasture phase. In 1995, paddocks were in at least their second year of cropping and denitrification may have occurred during the drought while the paddocks were bare.

Whilst there was no evidence from the crops sampled — or from observations of crops in the district in general — of negative effects on crop growth due to the slow rate of VAM colonisation, this could occur with more VAM dependent crops (Thompson 1987; Thompson 1994a; see discussion in §12.3.d.v). The lack of any evidence for the reduced VAM colonisation affecting crop growth is consistent with the results of the glasshouse trials reported in Chapter 7.

8.4. Summary of the Drought Results

- The drought in 1994 reduced VAM colonisation to negligible levels in most crops and severely retarded crop growth. The normally high level of VAM fungi on the alternative farms did not buffer the effect of the drought.

- The degree of reduction in VAM colonisation appeared to correlate to the degree of water stress the host plant was suffering.

- It was not determined whether the low colonisation levels were due to direct effects on the fungi or indirect effects mediated through the plant, however they did not appear to be due to high shoot P, at least on the alternative farms.

- The low colonisation levels during the drought indicated that VAM fungi were not playing a significant role in alleviating crop drought stress.

- The higher colonisation of the earlier sown, and less drought-affected, crops indicated that when assessing the effects of VAM fungi on host plant drought stress, the time that the stress is imposed, relative to the lifecycles of the fungi and the plant, must be considered.

- It appears that the 1994 drought reduced the levels of VAM inoculum in the soil, resulting in a slower rate of colonisation in 1995 cereal crops. It is unlikely that this affected crop growth.

Part D

Irrigated Dairy Pastures

Chapter Nine

The Dairy Farms Studied in this Project

Irrigated dairy farms were the second commodity production system examined in this project and this chapter contains background information on their environmental and management characteristics. The large number of dairy farms sampled allowed a more thorough investigation of broad ecological trends than was possible for the mixed farms, where only three farm pairs were sampled. However, it also meant that sampling on each individual farm was much more limited. Thus, in this chapter, detailed descriptions of each farm are not provided and instead, more general information is presented on the conventional and alternative farms.

9.1. Introduction

Two groups of farms were examined, 10 pairs of conventional/biodynamic farms located in NE Victoria and an additional nine pairs — where the alternative farms in each pair were either biodynamic or organic — located in other regions across SE Australia.

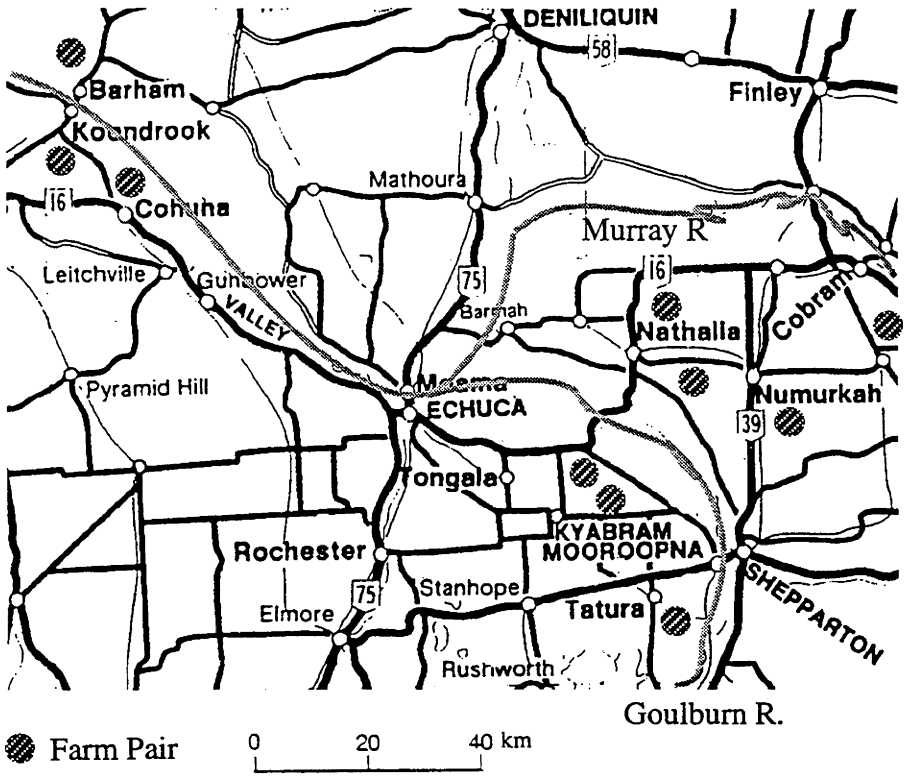
The dairy pastures were more intensively managed than the pastures on the mixed farms described in Chapter 6. High rates of N and P fertilisers were applied on the conventional farms and all farmers used irrigation to maintain pasture production over summer. In addition, unlike the annual pastures on the mixed farms, the dairy pastures were permanent (Plate 9.1) and not subjected to the regular disturbance of cropping.

9.2. Collaboration

All dairy farms sampled in this project were being examined as part of an established study, coordinated by Small from the Kyabram Dairy Centre. That study, jointly funded by the Dairy Research and Development Corporation (DRDC) and the Victorian Department of Agriculture, was titled "Alternative Farming Practices Applicable to the Dairy Industry" and ran from July 1991 to June 1994. During that time, 20 farms in NE Victoria were sampled up to five times and an additional 18 farms, located elsewhere around SE Australia, were sampled once. The study examined farm management strategies, soil and pasture characteristics, herd health and management, milk characteristics, milk production, pasture intake and farm financial status (Small *et al.* 1994a; Wynen 1994a). Thus, the dairy farms sampled during this project were selected by Small and much of the soil and pasture nutrient concentration information presented in Chapter 10 was collected by Small and co-workers and is summarised in Small *et al.* (1994a).

9.3. The Use of Paired Farms

In 1994 it was estimated that 27 dairy farms were under alternative management in Australia, with the majority being biodynamic (Small *et al.* 1994a). Many of these biodynamic dairy farms are located in the Goulburn and Murray River Valleys in NE Victoria and 10 of these farms, along with a conventional neighbour, were sampled during this project. Another nine alternatively managed dairy farms — three organic and six biodynamic — along with a conventional neighbour, located in other regions in SE Australia, were also sampled (Fig. 9.1). Thus, around 70% of alternatively managed dairy farms in Australia were sampled during this project.



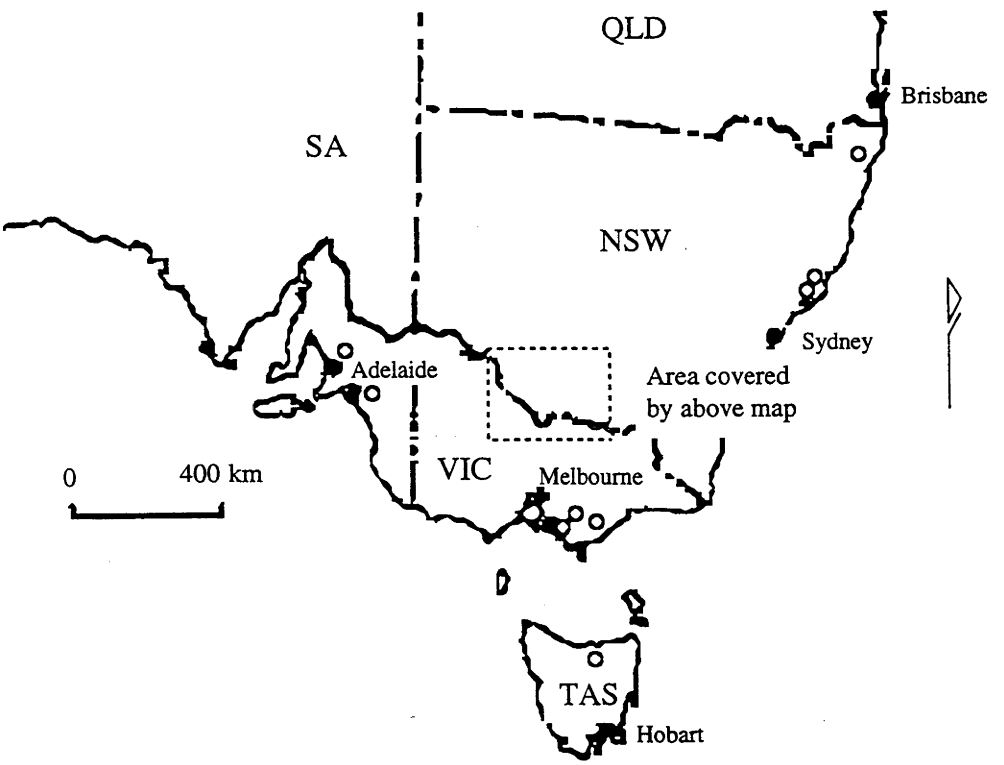


Figure 9.1. Location of the nine dairy farm pairs in SE Australia (open circles, lower map) and the ten pairs in NE Victoria (upper map)

Plate 9.1. Irrigated permanent pasture on a conventional dairy farm showing the three main pasture species: white clover (*Trifolium repens* L.), perennial rye grass (*Lolium perenne* L.) and paspalum (*Paspalum dilatatum* Poir).

Plate 9.2. An irrigated permanent pasture and laneway on a biodynamic farm in the Goulburn Valley, NE Victoria.

Selection of the conventional neighbour was based on soil type, regular fertiliser history, proximity to the biodynamic farm, herd breed, farm area and willingness to participate in the study (Small *et al.* 1994b).

9.4. Field Site and Farm Characteristics

9.4.a. NE Victorian Farms

(i) Field Site Characteristics

Twenty farms, 16 in the irrigation regions of the Goulburn River Valley in northern Victoria and four in the Murray River Valley in southern NSW, were sampled. These were located at Barham, Cohuna, Katandra West, Koondrook, Kyabram, Nathalia, Numurkah, Tallygaroopna and Tatura (Fig. 9.1 and Plate 9.2). While the 10 biodynamic farms represented 90% of farms in the area under biodynamic management, the conventional farms represented only 0.3% of dairy farms in the same area (Small *et al.* 1994b). All biodynamic farms had been biodynamic for at least three years and, on average, for 16 years.

Dairying is the enterprise that dominates these regions. Irrigation farms were created as part of state-sponsored closer settlement schemes during the 1920's and 1930's. The landscape is naturally flat and paddocks are flood-irrigated, with the flow of water across paddocks being regulated by check banks; contour banks around 0.5 m width and 50-100 mm high.

Monthly climate data for summer and winter is presented in Table 9.1. Cool temperatures limit pasture growth over winter and frosts generally occur between June and August. Annual rainfall is approximately 450 mm and annual evaporation 1600 mm. Irrigation water is applied from August to April — that is, spring to mid-autumn — and is equivalent to an additional 660 mm of rainfall. During summer, due to the erratic rainfall and high rates of evaporation, there is little pasture growth without irrigation. Overall, the climate is broadly similar to that of Ardlethan (Table 4.1), however, as the dairy farms were irrigated, rainfall is not so critical.

Table 9.1. Average climate data for Kyabram (elevation 100 m) in the Goulburn Valley, NE Victoria, in summer (December to February) and winter (June to August).

	Monthly rainfall (mm)	Monthly evaporation (mm)	Maximum temperature (°C)	Minimum temperature (°C)
Summer	36	260	29	21
Winter	40	39	14	3

The Goulburn Valley is characterised by red brown earth soil types situated on a vast and flat depositional riverine plain that extends into southern NSW. The topsoil is typically a loam, while the subsoil has a high clay content, usually > 50% (Doyle *et al.* 1996). The dense subsoil, which extends from 0.1 m to > 1.0 m depth, restricts root growth (Cock 1991) and leads to shallow penetration of irrigation water and surface waterlogging in unusually wet autumns or winter months. Recently, high water tables have become a problem and may result in constant waterlogging in the root-zone and a reduction in pasture growth. This may be exacerbated by a high salt content in the ground water (Doyle *et al.* 1996).

These soils have few nutritional problems, with the main deficiencies being P, N and S. The application of superphosphate on conventional farms is generally believed to overcome the P and S limitation to plant growth. However, while the legume component of the pasture supplies N through biological fixation, it is likely that N is limiting plant growth in most circumstances (Doyle *et al.* 1996).

Pastures on these farms are either irrigated perennial pastures or annual pastures. Only permanent irrigated perennial pastures were sampled during this project; these had not been cultivated for between eight and 40 years. These pastures comprised mainly white clover (*Trifolium repens* L.), perennial rye grass (*Lolium perenne* L.) and paspalum (*Paspalum dilatatum* Poir.) (Plate 9.1). On average in 1993, 8.5% of biomass consisted of clover, 32% paspalum, 45% other grasses and 13% weeds (Small *et al.* 1994a). Paspalum is regarded as a poor species for dairy production as it has low digestibility and produces little dry matter between April and October, but it is likely to be the highest yielding component of pasture between November and March (Doyle *et al.* 1996).

(ii) Farm Management

Conventional and biodynamic farm management were contrasted in section 2.3, the major difference being that biodynamic farmers do not apply synthetic fertilisers and rarely use synthetic chemical biocides. Differences between the conventional and biodynamic NE Victorian dairy farms are discussed below and some basic statistics presented in Table 9.2. To provide an indication of the variation present within each management system, selected information on individual farms is presented in Appendix 5.

The conventional and biodynamic farms had similar areas and herd sizes and did not differ significantly in the provision of cereal and hay supplements. Calves on the biodynamic farms had a higher parasite burden of worms and liverfluke, possibly leading to lower growth rates. Cows on the conventional farms were drenched up to six times annually, while on the biodynamic farms, drenches were used very occasionally only when an individual animal was sick. Conventional and biodynamic cows had

similar faecal parasite egg counts, suggesting the cows on the conventional farms were over-treated with drench (McDonald *et al.* 1994). Milk production was higher on the conventional farms.

Table 9.2. Selected average characteristics of the 10 conventional and biodynamic farms sampled in NE Victoria (Small *et al.* 1994a; Small and McDonald 1993; Small *et al.* 1994b; Wynen 1994a).

	Conventional	Biodynamic
Effective hectares	62	62
Cows milked	134	117
Cereal supplements (kg cow^{-1} $year^{-1}$)	550	320
Artificial insemination (% herd)	67	67
Cows (treatment $animal^{-1}$ $year^{-1}$)		
- stomach worms	0.9	0.03
- liver fluke	1	0.1
N fertilisers (kg ha^{-1} $year^{-1}$ of N)	17	0
P fertilisers (kg P ha^{-1} $year^{-1}$ of P)	27	0
Milk production (l ha^{-1} yr^{-1})	9 060	6 740
Total water use (ML $year^{-1}$)	410	333
Irrigation interval (days)	8	14
Rate of return to resources ($ ha^{-1})	793	114

All conventional farms had at least a 15 year history of regular superphosphate additions, while biodynamic farmers had not applied significant amounts of soluble P fertilisers for, on average, 17 years (Small *et al.* 1994b). Lime was added at similar rates to farms under both management strategies. Nitrogen fertilisers were not used by biodynamic farmers.

Financially, total costs were 33-40% lower on the biodynamic farms, while costs for producing a litre of milk were 3-15% higher than on the conventional farms. Income on the biodynamic farms was approximately 60% of that on the conventional (Wynen 1994a). If the cash costs are adjusted for changes in trading stock, depreciation, family labour, interest and rent payments, a measure is gained of the return to resources; this was 500% greater for the conventional farms (Wynen 1994a).

All farms were flood irrigated. Biodynamic farms had a longer irrigation interval and more water was applied at each irrigation, however, their total water use was less. The longer irrigation interval does not result in wilting of pasture, which Small *et al.* (1994b) suggest may indicate better soil structure on the biodynamic farms. Research on one farm pair found more favourable soil physical characteristics on the biodynamic farm (Lytton-Hitchins 1992; Lytton-Hitchins *et al.* 1994).

Other soil and pasture characteristics are described in Small *et al.* (1994b). Soil concentrations of organic carbon, total Kjeldahl N, Cu, Zn, Se, humic acid, fulvic acid and pH did not differ between the two systems. However, half the biodynamic farms had soil sulfur levels which were low or marginal and conventional farms had higher levels of available Cd (Small *et al.* 1994b). Extractable P and total P were consistently higher for conventional soils, particularly in the soil mat — the dead organic matter on the soil surface — and top 50 mm of soil. Salinity levels on the biodynamic farms were not high enough to reduce growth of salt-sensitive plants, but on the conventional farms salinity was high enough to marginally effect the growth of white clover (Small*et al.* 1994b). Soil microbial biomass did not differ between the two systems, but the conventional farms had a greater number and biomass of earthworms, possibly due to the greater amounts of manure produced. Pasture P was higher on the conventional farms and was marginal for optimum plant growth on the biodynamic farms. Sulfur, Ca and Na concentrations were occasionally lower in pasture from biodynamic farms. The proportions of clover, rye grass, paspalum and weeds in pasture varied greatly between farms, but did not differ between conventional and biodynamic farms.

9.4.b. Other Dairy Farms Sampled in S E Australia

(i) Field Site Characteristics

Permanent irrigated pastures on nine pairs of dairy farms located in other areas around SE Australia were also sampled during this project (Fig. 9.1). Limited background information was available on this second set of farms. Annual rainfall varied from 750-3500 mm, compared with 450 mm for the NE Victorian farms. Soil type varied from fine sandy loam to fine sandy clays, clay loams, light medium clays and medium clays (Small *et al.* 1994a).

(ii) Farm Management

General management characteristics are outlined in Table 9.3. Of the alternatively managed farms, three were organic and the remainder biodynamic. Conventional and alternative farms generally did not differ in farm size, herd size, or level of cereal supplementation, while conventional cows were again more likely to receive drenches. Compared to the NE Victorian farms, average farm size was larger and stocking rate was lower, with herd size ranging from 42-270. Application of P and N fertilisers was much higher on the conventional farms and milk production was greater on the conventional farms; although, under both management systems it was significantly lower than on the NE Victorian farms. Pasture composition was similar to on the NE Victorian farms (Small *et al.* 1994a).

Table 9.3. Characteristics of conventional, organic and biodynamic farms sampled in other regions of SE Australia (Small *et al.* 1994a); economic analysis was not performed on these farms.

	Conventional	Alternative
Effective hectares	93	83
Cows milked	118	110
Cereal supplements (kg cow $^{-1}$ year^{-1})	690	580
Artificial insemination (% herd)	66	73
Cows (treatment animal^{-1} year^{-1})		
- stomach worms	0.4	0.02
- liver fluke	0	0.02
N fertilisers (kg ha^{-1} year^{-1} of N)	29	5
P fertilisers (kg ha^{-1} year^{-1} of P)	35	0
Milk production (l ha^{-1})	5 430	4 460
Irrigation interval (days)	7	10

9.5. Other Sources of Information on the Dairy Farms

There are a number of additional sources of information on these farms, including preliminary reports on the NE Victorian farms (Small and McDonald 1993; Small *et al.* 1994b), the final project report on the NE Victorian and other SE Australian farms (Small *et al.* 1994a), a more detailed report on animal health on the Victorian farms (McDonald *et al.* 1994), details of the soil structure on one pair of farms at Nathalia (Lytton-Hitchins 1992; Lytton-Hitchins *et al.* 1994), the P budget on one pair of farms at Barham (Parker 1992), an examination of soil and plant root characteristics on one pair of farms (Cock 1991) and an economic analysis of the NE Victorian farms (Wynen 1994a).

Chapter Ten

Factors Affecting VAM Colonisation Levels and Plant Nutrient Concentrations in Irrigated Perennial Dairy Pastures

This chapter contains the results from field surveys conducted on permanent irrigated dairy pastures on conventional/alternative farm pairs in SE Australia. The first was a single sampling of 10 farm pairs in NE Victoria in March 1993, the second a single sampling of nine farm pairs located in other regions of SE Australia in March 1994 and the third consisted of sampling three farm pairs in NE Victoria six times between March 1993 and November 1996. Farm locations and general farm management practices were described in Chapter 9. Results are grouped into three main areas: examination of relationships between VAM colonisation levels and soil and plant parameters; variation in VAM colonisation over time; and VAM spores A detailed analysis of these farms was carried out between mid-1991 and mid-1994 by Small and other researchers from the Kyabram Dairy Centre. Small provided much of the data presented in this chapter on nutrient concentrations in soil and pasture; this data is summarised in Small *et al.* (1994a). Plant growth was assessed in glasshouse trials, presented in Chapter 11, due to the complex mixed-species nature of the pasture and its constant grazing by cattle.

10.1. Aims

The aims of the research reported in this chapter were to investigate the following questions.

- What were the major factors influencing VAM colonisation levels in dairy pastures?
    - Were these consistent for the three dominant pasture species; white clover (*Trifolium repens* L.), perennial rye grass (*Lolium perenne* L.) and paspalum (*Paspalum dilatatum* Poir.)?
    - Were these consistent across the variety of locations sampled in SE Australia?
- What were the major factors influencing the frequency of *Rhizobium* nodules on clover roots?
- What were the major factors influencing pasture P concentrations?
- Did the level of VAM colonisation remain stable over 3.5 years?
- Did the numbers and types of VAM spores differ between conventional and alternative farms?
- Were the relationships between VAM fungi and soil and plant nutrient concentrations similar on the conventional and alternative farms?

10.2. Methods

All sampling was of grazed perennial pasture, which had been established for at least seven years, on paired alternative/conventional dairy farms. In NE Victoria, eight farm pairs were located in the Goulburn River Valley and two in the Murray River Valley. Farm pairs in other regions around SE Australia were located in New South Wales (3), Gippsland in Victoria (3), Tasmania (1) and South Australia (4). In NE Victoria, the alternative farm was always biodynamic, while for the nine farm pairs in other regions of SE Australia, the alternative farm was organic or biodynamic. These locations were shown in Figure 9.1.

The 10 NE Victorian farm pairs were sampled in March 1993 and the 10 farm pairs in other areas of SE Australia in March 1994. Three farm pairs (Pairs A, B and C) in the Goulburn River Valley in NE Victoria were resampled in June 1993, November 1993, January 1994, November 1994 and November 1996 to construct a time series of VAM colonisation and to more closely investigate some of the relationships found in the March 1993 sampling. A paddock on the conventional farm in Pair A which had received no P fertiliser for 10 years was also sampled at these later times. Whenever possible, the same paddock on each farm was repeatedly sampled.

Root samples to be assessed for VAM colonisation were collected from 20 sites in one paddock on each farm (§3.2). On the NE Victorian farms in March 1993, roots

were collected from white clover and one of the major grass species present, rye grass or paspalum. On the farms in other areas of SE Australia in March 1994, only clover was sampled, as the dominant grass species varied between locations. In the time series, clover and rye grass were sampled at each date, while paspalum — which is dormant over winter — was only sampled in January 1994. In addition, in November 1994, clover and rye grass roots were collected from two biodynamic farms to investigate the effect of check banks — low contour banks approximately 50-100 mm high and 0.5 m wide which aid even distribution of irrigation water — and manure on VAM colonisation. In one paddock, at 20 sites, pairs of samples were taken from check banks and adjacent irrigated pasture. In a second paddock, paired samples were collected at 20 sites from under pasture where cattle manure had been deposited around four weeks previously — although the manure had largely decomposed, these areas were obvious as the cattle had not grazed them — and adjacent pasture.

Roots were stained and VAM (%) assessed (§3.5). Colonisation intensity was also assessed on the March 1993 sampling of the NE Victorian farms and March 1994 sampling of farms in other regions of SE Australia (§3.5.c). The frequency of *Rhizobium* nodules was assessed on clover (§3.4.e) from the six farms sampled in January 1994.

On the NE Victorian farms, soil cores were taken to 100 mm (without the soil mat) from 20 sites in each of two paddocks on each farm in October 1991, March 1993 and March 1994 by Small. The nine farm pairs in other areas of SE Australia were similarly sampled in March 1994. Soil samples were analysed by Small for Olsen P (Olsen *et al.* 1954), total P in the Olsen extract, Colwell P (Colwell 1963) and total Kjeldahl N (§3.6). In addition, in November 1994 and November 1996, samples of soil to 100 mm were taken by the author from 15 sites in a paddock from each of the six time series farms and bulked. One subsample from each farm was sent to Wesfarmers CSBP (Perth, Western Australia) for determination of extractable P (Olsen) and total N.

Whole pasture samples (all plant species combined) were taken from 60 sites in each of two paddocks on each farm in March 1993 on the NE Victorian farms and March 1994 on the farms in other regions in SE Australia by Small. Subsamples of whole pasture were analysed for mineral concentrations (§3.3.b) by Small. In addition, in January 1994, samples of pasture were collected by the author from 15 sites in one paddock on each of the six time series farms. The pasture was separated into clover, rye grass and paspalum and shoots (stems and leaves) analysed for P and N concentrations (§3.3.b).

Clover and rye grass roots from the six farms sampled in October 1993 and clover roots from January 1994 were bulked at the paddock level, owing to their small size, and P and N concentrations determined (§3.3.b)

In November 1994, samples of soil to 100 mm were collected from five sites in eight paddocks on the six time series farms and stored at 4°C. The conventional paddock which had received no P fertiliser was also sampled and a second paddock was sampled on its biodynamic neighbour. VAM spores were extracted, counted and classified into six types, but identification was not attempted (§3.5.c); accurately identifying VAM species from field samples of spores may be virtually impossible to achieve and may not provide useful information (J. Morton, 1996, pers. comm.).

Results are presented in three sections: the relationships between VAM colonisation levels and soil and plant parameters; the variation in VAM colonisation over time; and VAM spores. Results are initially presented graphically, or in tables, as mean values with standard errors (§3.9). The statistical relationships between the variables are explored with particular emphasis on investigating both which factors were most strongly influencing VAM colonisation levels and whether the relationships between various soil, plant and VAM measures were similar on the conventional and alternative farms. Much of the data used in the statistical analysis was provided by Small and, unlike the data supplied by Derrick in Chapter 5, was not collected from the same sites in each paddock as the VAM samples. Thus, the mean value from each farm was used in the statistical analyses. The time series data is presented graphically, but not analysed statistically.

10.3. Results

10.3.a. The Relationships between VAM Colonisation Levels and Soil and Pasture Nutrient Concentrations

(i) Soil and Pasture Nutrient Concentrations

Colwell and Olsen extractable P were higher on the conventional farms (Table 10.1). On the NE Victorian farms, extractable P on the conventional farms was more than double that on the biodynamic farms. The difference was less, but still substantial, on the farms from other regions in SE Australia. Total P in the Olsen extract was greater on the conventional farms, but the difference was much less than for extractable P. Soil total N and soil pH did not differ consistently between the alternative and conventional farms. Being a more natural group for comparison, variation between farms was less for the NE Victorian farms.

Table 10.2 contains the concentration of P and N in whole pasture shoots. Pasture P was 30-46% higher in the conventional pasture. Pasture N was similar in pasture from the conventional and biodynamic farms in NE Victoria, but on the farms from other regions of SE Australia, tended to be higher in the conventional pasture.

Table 10.1. Olsen and Colwell extractable P, total P in the Olsen extract, total N and pH of soil on conventional and alternative dairy farms in NE Victoria (March 1993) and other regions in SE Australia (March 1994); mean *(s.e.m.)*, n=10 farm pairs (NE Victoria), n=9 farm pairs (other regions). Data from Small *et al.* (1994a).

	NE Victoria		Other regions in SE Australia	
	Conventional	Alternative	Conventional	Alternative
Olsen extractable P ($\mu g\ g^{-1}$)	19.2 *(3.4)*	8.4 *(1.6)*	20.6 *(3.1)*	14.5 *(6.3)*
Colwell extractable P ($\mu g\ g^{-1}$)	87.7 *(11.6)*	36.4 *(5.4)*	78.0 *(11.7)*	50.6 *(17.5)*
Total P in the Olsen extract ($\mu g\ g^{-1}$)	35.8 *(2.8)*	26.9 *(2.9)*	89.6 *(9.9)*	68.1 *(10.8)*
Total N ($\mu g\ g^{-1}$)	3500 *(30)*	3600 *(40)*	5800 *(90)*	5800 *(50)*
pH	6.3 *(0.2)*	6.0 *(0.1)*	5.7 *(0.1)*	6.1 *(0.2)*

Table 10.2. The concentration of P and N in whole pasture shoots (all species combined) from conventional and alternative dairy farms in NE Victoria (March 1993) and other regions in SE Australia (March 1994); mean *(s.e.m.)*, n=10 farm pairs (NE Victoria), n=9 farm pairs (other regions). Data from Small *et al.* (1994a).

	NE Victoria		Other regions in SE Australia	
	Conventional	Alternative	Conventional	Alternative
Pasture P concentration	0.35 *(0.02)*	0.24 *(0.01)*	0.35 *(0.02)*	0.27 *(0.02)*
Pasture N concentration	1.99 *(0.07)*	2.11 *(0.08)*	2.52 *(0.13)*	2.21 *(0.16)*

(ii) Level of VAM Colonisation in Pasture

The percentage of clover root length colonised by VAM fungi on the NE Victorian farms in March 1993 is shown in Figure 10.1.a. The pairs have been ordered to emphasise the variation on the conventional farms. Pairs 1-9 of the NE Victorian farms are in the Goulburn River Valley and 9-10 are in the Murray River Valley. VAM colonisation was greater on the alternative farms than the conventional farms, with the exception of pair 8, and colonisation was much more variable on the conventional farms. Overall, VAM colonisation in clover appeared to plateau at around 80% of root length. On the farms in other regions of SE Australia (Fig. 10.1.b), VAM colonisation levels were higher on the alternative farms, with two exceptions located in northern NSW at Rollands Plains and Comboyne.

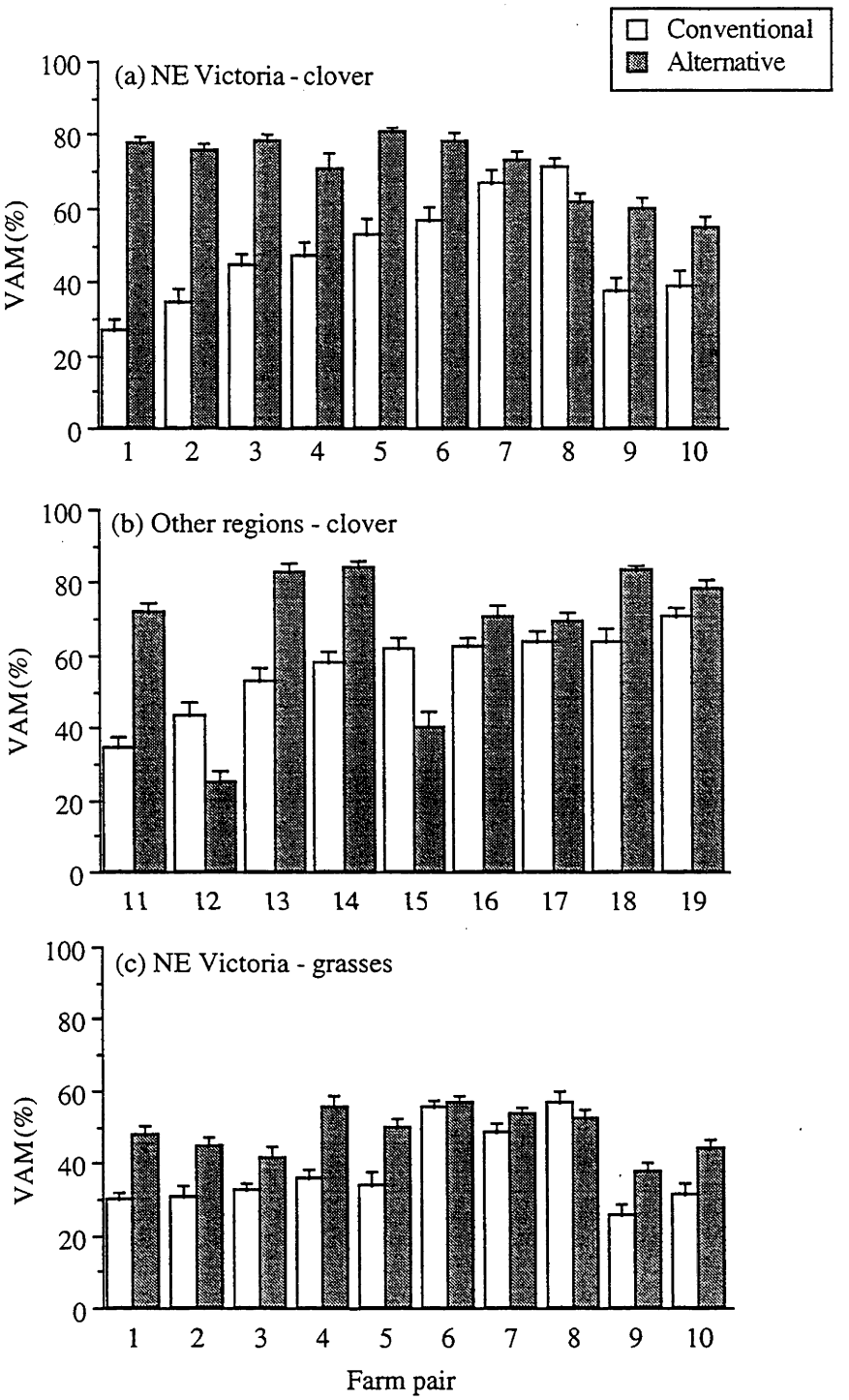


Figure 10.1. Percentage of root length colonised by VAM fungi on paired conventional and alternative farms for a) clover on NE Victorian farms in March 1993, b) clover on farms in other regions of SE Australia in March 1994 and c) grasses on NE Victorian farms in March 1993; mean ± s.e.m., n=20. Alternative farms in pairs 13, 14, 17 and 19 were organic; the rest were biodynamic.

VAM colonisation of the predominant grass species on the NE Victorian farms was also higher on the alternative farms, with the exception of pair 8 (Fig. 10.1.c). However, colonisation was generally lower than for the clover and the differences between conventional and alternative farms were less. A comparison of colonisation levels in clover, rye grass and paspalum in six paddocks in January 1994 is presented in Figure 10.2. The paspalum and rye grass had similar colonisation levels, which did not vary as much between farms as the colonisation of clover.

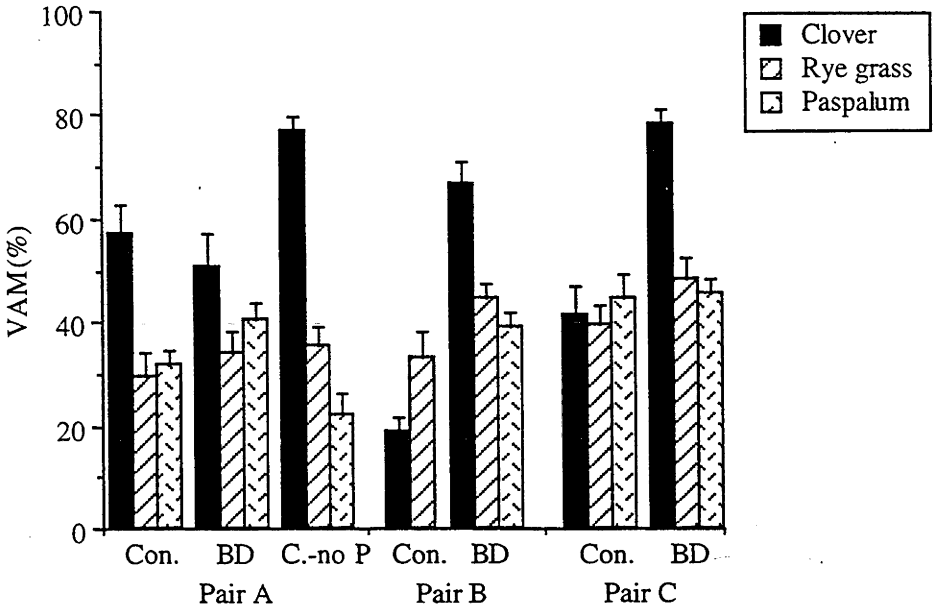


Figure 10.2. The percentage of clover, rye grass and paspalum root length colonised by VAM fungi on three farm pairs in NE Victoria in January 1994; mean ± s.e.m., n=15. Farm management strategy is indicated; conventional (Con.), biodynamic (BD), or conventional with no P fertiliser additions (C.-no P).

Table 10.3 summarises the VAM colonisation levels and VAM intensity under each farm management strategy for the two groups of farms. On the NE Victorian farms, VAM (%) was higher on the alternative farms than on the conventional farms for both clover (48% higher) and grasses (26% higher). For the clover, a greater proportion of colonisation was in the two higher intensity categories on the alternative farms than on the conventional farms. However, for the grasses, there was no difference between management strategies in the proportion of colonisation in each intensity category. On the farms located in other regions around SE Australia, VAM (%) was slightly higher on the alternative farms and there was little difference in VAM intensity.

Table 10.3. Average levels of VAM colonisation on conventional and alternative farms. Total percentage of root length colonised and the percentage of colonised root length which fell into three intensity categories: less than half the root cortex colonised (low); half to all the root cortex filled with continuous colonisation of low to medium density (medium); and dense colonisation of the entire root cortex (high); mean *(s.e.m.)*, n=20.

Location	Farm management strategy	Pasture species	Total colonisation (%)	Low intensity (%)	Medium intensity (%)	High intensity (%)
NE Victoria	Conventional	clover	48 *(4.5)*	54 *(4.8)*	37 *(2.5)*	9 *(2.8)*
	Alternative		71 *(2.9)*	38 *(4.3)*	44 *(1.9)*	18 *(3.2)*
	Conventional	grasses	38 *(3.6)*	51 *(3.9)*	43 *(2.7)*	6 *(1.3)*
	Alternative		48 *(2.0)*	47 *(2.8)*	45 *(1.8)*	8 *(1.2)*
Other	Conventional	clover	57 *(3.8)*	66 *(2.4)*	31 *(2.1)*	3 *(0.8)*
	Alternative		67 *(6.9)*	53 *(6.8)*	38 *(4.7)*	9 *(2.5)*

(iii) Relationships between VAM Colonisation Levels, Soil and Pasture Nutrient Concentrations, and Farm Management Strategy

Three types of statistical analyses were made: ANOVAs to examine the effect of farm location and farm management strategy; regression analyses to examine the interactions between continuous biological or soil factors; and stepwise regressions to examine whether location and, in particular, farm management strategy, explained variation in the data in addition to that explained by the continuous soil and biological variables. Simple regressions of farm means were summarised graphically. ANOVA and regression analyses were made separately. The strong correlation of both farm location and farm management strategy with biological and soil variables — such as soil extractable P — made it impossible to construct a model which included these two sets of variables and gave meaningful results.

In the ANOVAs, two classifications of location were trialed: separation into NE Victorian farms (n=20) and farms from other regions (n=18); and separation into Goulburn River Valley (n=16), Murray River Valley (n=4), New South Wales (n=6), Gippsland (n=6), Tasmania (n=2) and South Australia (n=4). The second classification was used as it explained more variation in the data. Table 10.4 contains the results from ANOVAs where location and farm management strategy were fitted. Location affected soil total N, soil total P and soil pH. Farm management strategy affected soil extractable P, pasture P and VAM (%) in clover.

Table 10.4. Summary of the significant predictor variables and their level of significance when soil and biological parameters measured on 38 dairy farms were fitted with location (Goulburn Valley, Murray River Valley, New South Wales, Gippsland, Tasmania, South Australia) and farm management strategy (conventional, alternative). There were no significant interactions between location and management strategy. All data, except VAM (%), from Small *et al.* (1994a).

Factor	Location	Farm Management Strategy
Soil extractable P (Olsen)	-	0.03
Soil total P	<0.0001	-
Soil total N	<0.0001	-
soil pH	0.02	-
Pasture P concentration	-	<0.0001
Pasture N concentration	-	-
Clover VAM (%)	-	0.0004

Table 10.5 presents results from regressions of the relationships between soil variables, biological variables and VAM colonisation using data from all 38 farms. VAM colonisation of both clover and grasses was strongly negatively correlated with pasture P and log soil extractable P (Fig. 10.3). The correlation was identical for Olsen or Colwell extractable P, which were strongly correlated (Fig. 10.4). Clover VAM intensity was also negatively correlated with log soil extractable P. Log VAM intensity correlated strongly with the VAM (%) (Fig. 10.5), with log soil extractable P also exerting a influence independently of its effect on VAM colonisation. Pasture P was strongly correlated with log soil extractable P (Fig. 10.6).

Table 10.5. Results of regressions of data collected on the NE Victorian farms in March 1993 and the farms in other regions of SE Australia in March 1994. Parameters included are the percentage of root length colonised by VAM fungi, VAM intensity, pasture P (%) and Olsen (O) and Colwell (C) soil extractable P ($\mu g\ g^{-1}$). Soil and pasture P data from Small *et al.* (1994a). VAM colonisation in grasses was available only for the NE Victorian farms. Soil total P and N, pasture N, and soil pH did not exert a significant influence in any of the models.

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
Clover VAM (%)	full model	-	-	21.7	<0.0001	0.36	38
	intercept	102.5	9.3	12.1[t]	<0.0001		
	pasture P	-141.6	30.4	21.7	<0.0001		
Grass VAM (%)	full model	-	-	4.5	0.047	0.16	20
	intercept	64.1	10.1	6.4[t]	<0.0001		
	pasture P	-74.0	34.8	4.5	0.047		
Clover VAM intensity	full model	-	-	31.7	<0.0001	0.45	38
	intercept	222.9	21.6	10.3[t]	<0.0001		
	log extractable P (O)	-108.3	19.2	31.7	0.0000		
Clover VAM (%)	full model	-	-	26.2	<0.0001	0.41	38
	intercept	100.7	8.1	12.4[t]	<0.0001		
	log extractable P (O)	-37.0	7.2	26.2	<0.0001		
Clover VAM (%)	full model	-	-	22.0	0.0000	0.36	38
	intercept	126.8	14.3	8.9[t]	<0.0001		
	log extractable P (C)	-38.5	8.2	22.0	0.0001		
Soil extractable P (O)	full model	-	-	150.9	<0.0001	0.80	38
	intercept	-0.68	1.54	-0.44[t]	0.66		
	soil extractable P (C)	0.25	0.02	150.9	<0.0001		
Log clover VAM intensity	full model	-	-	91.8	<0.0001	0.83	38
	intercept	1.56	0.13	12.2[t]	<0.0001		
	Clover VAM (%)	0.009	0.001	73.4	<0.0001		
	log extractable P (O)	-0.16	0.06	5.9	0.02		
Pasture P	full model	-	-	56.1	<0.0001	0.60	38
	intercept	0.09	0.03	3.0[t]	0.005		
	log extractable P (O)	0.19	0.03	56.1	<0.0001		

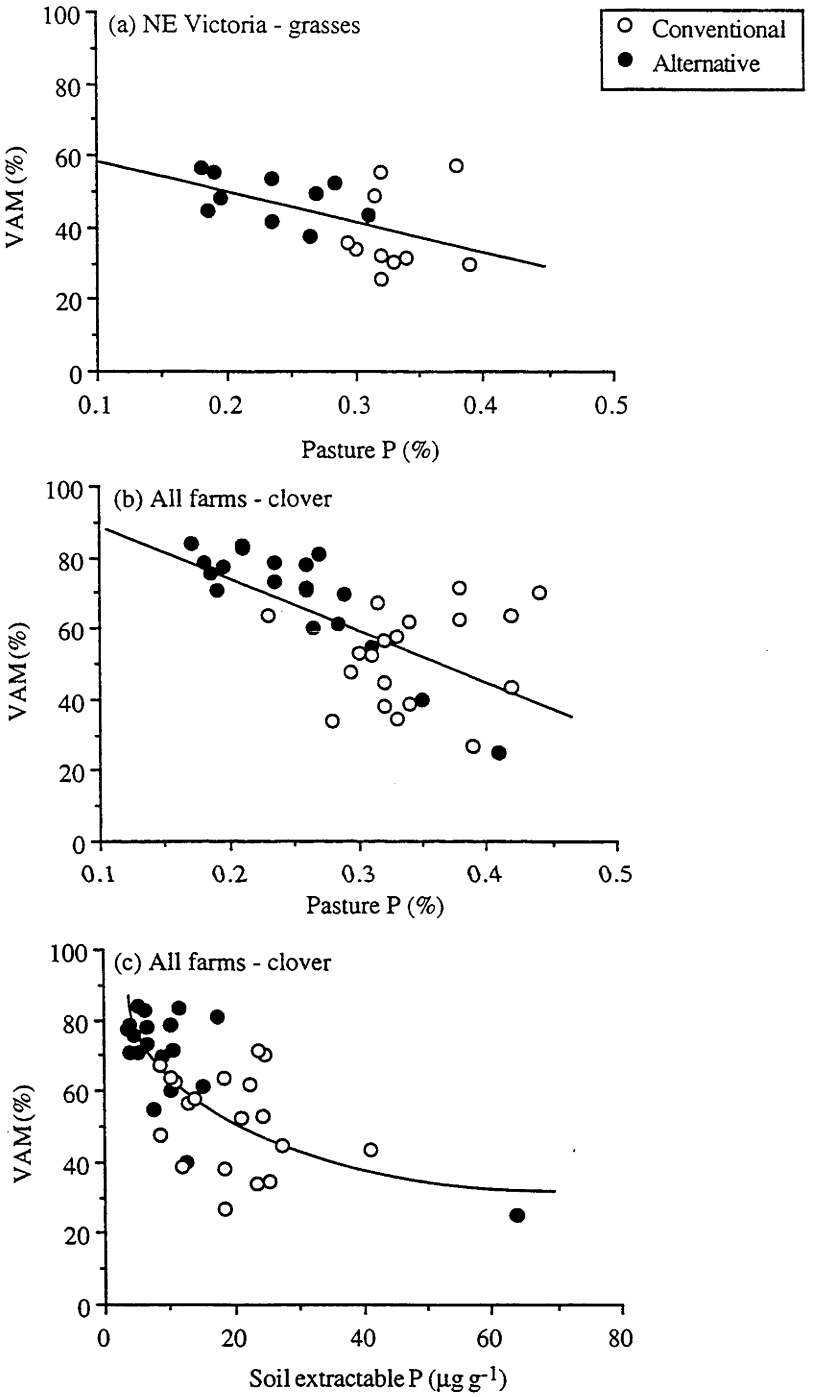


Figure 10.3. Simple regressions between a) percentage of grass root length colonised by VAM fungi and pasture P on NE Victorian farms in March 1993 (r^2=0.20), b) percentage of clover root length colonised by VAM fungi and pasture P on NE Victorian farms in March 1993 and farms in other regions of SE Australia in March 1994 (r^2=0.38) and c) percentage of clover root length colonised by VAM fungi and log soil extractable P (Olsen) on NE Victorian farms in March 1993 and other farms in March 1994 (r^2=0.42). Farm management strategy (conventional, alternative) is indicated. Each point represents the average from 20 sites in one paddock. Pasture and soil P concentrations from Small *et al.* (1994a).

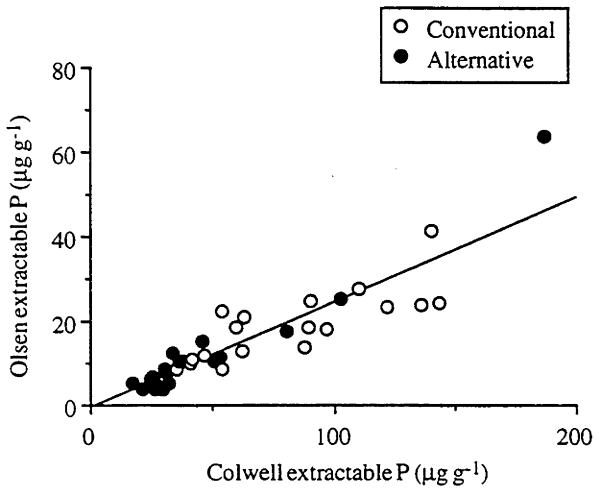


Figure 10.4. Relationship between Olsen extractable P and Colwell extractable P on the NE Victorian farms in March 1993 and the farms in other regions of SE Australia in March 1994 (r^2=0.81). The relationship can be expressed as *Olsen = -0.68 + 0.25.Colwell.* Each point represents the average from 20 sites in one paddock. Farm management strategy (conventional, alternative) is indicated. Data from Small *et al.* (1994a)

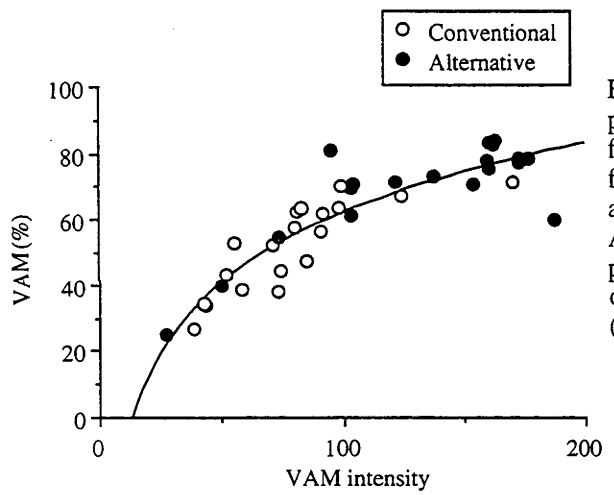


Figure 10.5. Relationship between the percentage of root length colonised by VAM fungi and log VAM intensity using data from the NE Victorian farms in March 1993 and the farms in other regions of SE Australia in March 1994 (r^2=0.81). Each point represents the average from 20 sites in one paddock. Farm management strategy (conventional, alternative) is indicated.

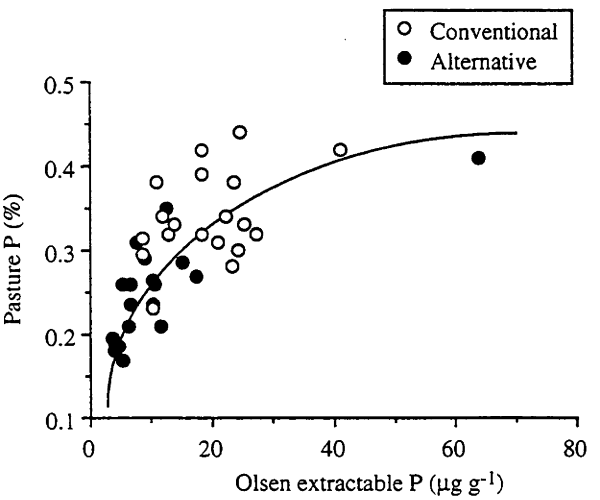


Figure 10.6. Relationship between pasture P and log soil extractable P (Olsen) using data from the NE Victorian farms in March 1993 and the farms in other regions of SE Australia in March 1994 (r^2=0.61). Each point represents the average from 20 sites in one paddock. Farm management strategy (conventional, alternative) is indicated. Data from Small *et al.* (1994a).

To more closely examine the relationships between plant nutrient concentrations and VAM (%), pasture samples collected in January 1994 from three farm pairs were separated into clover, rye grass and paspalum, and shoot P and N determined. VAM colonisation levels, *Rhizobium* nodulation in clover, and clover root P and N were measured on root samples collected at the same sites. Clover and rye grass roots collected from the same paddocks in October 1993 were also analysed for P and N.

Clover and rye grass shoot P tended to be higher on the conventional farms, while the paspalum shoot P was quite variable and showed no clear trends (Table 10.6). Shoot N in all species showed no consistent differences between conventional and biodynamic farms. A preliminary examination was made of root P and N concentrations (Table 10.6); as samples were bulked no indication of variation is given. Root P was markedly higher on the conventional farms, while root N showed no clear trend. *Rhizobium* nodules occurred more frequently on the conventional farm in Pairs B and C, but this trend was not evident in Pair A.

Table 10.6. Various measures from conventional (Con.), and biodynamic (BD) farms, as well as a conventional paddock which had received no P fertiliser for 10 years (Con.-no P), sampled in NE Victoria in January 1994: clover, rye grass and paspalum shoot P and N; clover root N and P (measures made on bulked samples); and *Rhizobium* nodules; mean *(s.e.m.)*, n=15.

	Pair A			Pair B		Pair C	
	Con.	BD	Con.-no P	Con.	BD	Con.	BD
Clover shoot P (%)	0.32 *(0.01)*	0.29 *(0.01)*	0.33 *(0.01)*	0.39 *(0.01)*	0.33 *(0.01)*	0.32 *(0.01)*	0.29 *(0.01)*
Clover shoot N (%)	3.43 *(0.07)*	3.79 *(0.03)*	3.61 *(0.09)*	3.51 *(0.06)*	3.61 *(0.06)*	3.53 *(0.07)*	3.57 *(0.06)*
Rye grass shoot P (%)	0.46 *(0.03)*	0.35 *(0.01)*	0.32 *(0.03)*	0.50 *(0.01)*	0.28 *(0.02)*	0.57 *(0.03)*	0.34 *(0.02)*
Rye grass shoot N (%)	2.41 *(0.09)*	2.05 *(0.07)*	1.89 *(0.07)*	1.97 *(0.08)*	2.29 *(0.07)*	2.23 *(0.10)*	1.84 *(0.07)*
Paspalum shoot P (%)	0.30 *(0.01)*	0.40 *(0.01)*	0.25 *(0.02)*	-	0.31 *(0.02)*	0.32 *(0.01)*	0.32 *(0.02)*
Paspalum shoot N (%)	2.04 *(0.11)*	2.21 *(0.06)*	2.48 *(0.12)*	-	2.57 *(0.06)*	3.36 *(0.09)*	2.38 *(0.07)*
Clover root P (%)	-	0.15	0.16	0.30	0.18	0.26	0.16
Clover root N (%)	-	1.58	2.05	2.00	1.96	2.37	1.74
Rhizobium nodules (number 100 mm^{-1} roots)	4.4 *(0.3)*	4.5 *(0.3)*	3.5 *(0.4)*	6.0 *(0.3)*	3.3 *(0.2)*	6.3 *(0.5)*	4.4 *(0.4)*

Table 10.7 presents the results of statistical analyses of some of the data presented in Table 10.6. The analyses use data from individual sites and, therefore, differ from those presented in Table 10.5 which used only paddock means. Some relationships in Table 10.7 are presented as simple regressions of paddock means in Figures 10.7 and 10.8. Relationships which involved parameters only available as paddock means — root nutrient concentrations and soil extractable P — are also included in Figures 10.7 and 10.8.

Clover VAM (%) was negatively correlated with shoot P (Table 10.7 and Fig. 10.7.a). As with the analyses of all farms (Table 10.5), shoot N did not exert a significant influence in the model. Note that the amount of variation explained when site data was used (r^2 = 0.24, Table 10.7) was lower than when only paddock means were used (r^2 = 0.43, Fig. 10.7.a). VAM colonisation in the two grasses was not strongly correlated with shoot nutrient concentrations (Table 10.7 and Fig. 10.7.b).

Table 10.7. Results of statistical analysis of data collected from 10-15 sites in seven paddocks on three farm pairs in NE Victoria in January 1994. Parameters included are the percentage of root length colonised by VAM fungi, shoot P (%) and frequency of *Rhizobium* nodules (nodules 100 mm^{-1} of roots). Pasture N did not exert a significant influence in any of the models.

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio or t-test	Prob.	r^2	n
Clover VAM (%)	full model	-	-	30.7	<0.0001	0.24	94
	intercept	140.8	15.6	9.0^t	<0.0001		
	clover shoot P	-263.5	47.6	30.7	<0.0001		
Rye grass VAM (%)	full model	-	-	3.7	0.06	0.03	96
	intercept	48.4	5.3	9.1^t	<0.0001		
	rye grass shoot P	-24.4	12.7	3.7	0.06		
Paspalum VAM (%)	full model	-	-	5.8	0.02	0.06	79
	intercept	21.8	7.2	3.0^t	0.003		
	paspalum shoot P	52.6	21.9	5.8	0.02		
Clover nodules	full model	-	-	4.3	0.04	0.03	95
	intercept	2.3	1.1	2.0^t	0.05		
	clover shoot P	7.2	3.5	4.3	0.04		

Other relationships involving VAM colonisation are shown in Figure 10.7.c-f. Clover VAM (%) was strongly negatively correlated with root P (Fig. 10.7.c), a trend not as strong for rye grass (Fig. 10.7.d). VAM colonisation in both the clover and the grasses was strongly correlated with soil extractable P measured in March (Fig. 10.7.e, f). All the relationships shown in Figure 10.7 were consistently stronger and steeper for clover than for the grasses. The frequency of clover root nodules was positively correlated with plant P concentrations, in particular root P (Table 10.7 and Fig. 10.8); N concentrations did not exert a significant influence.

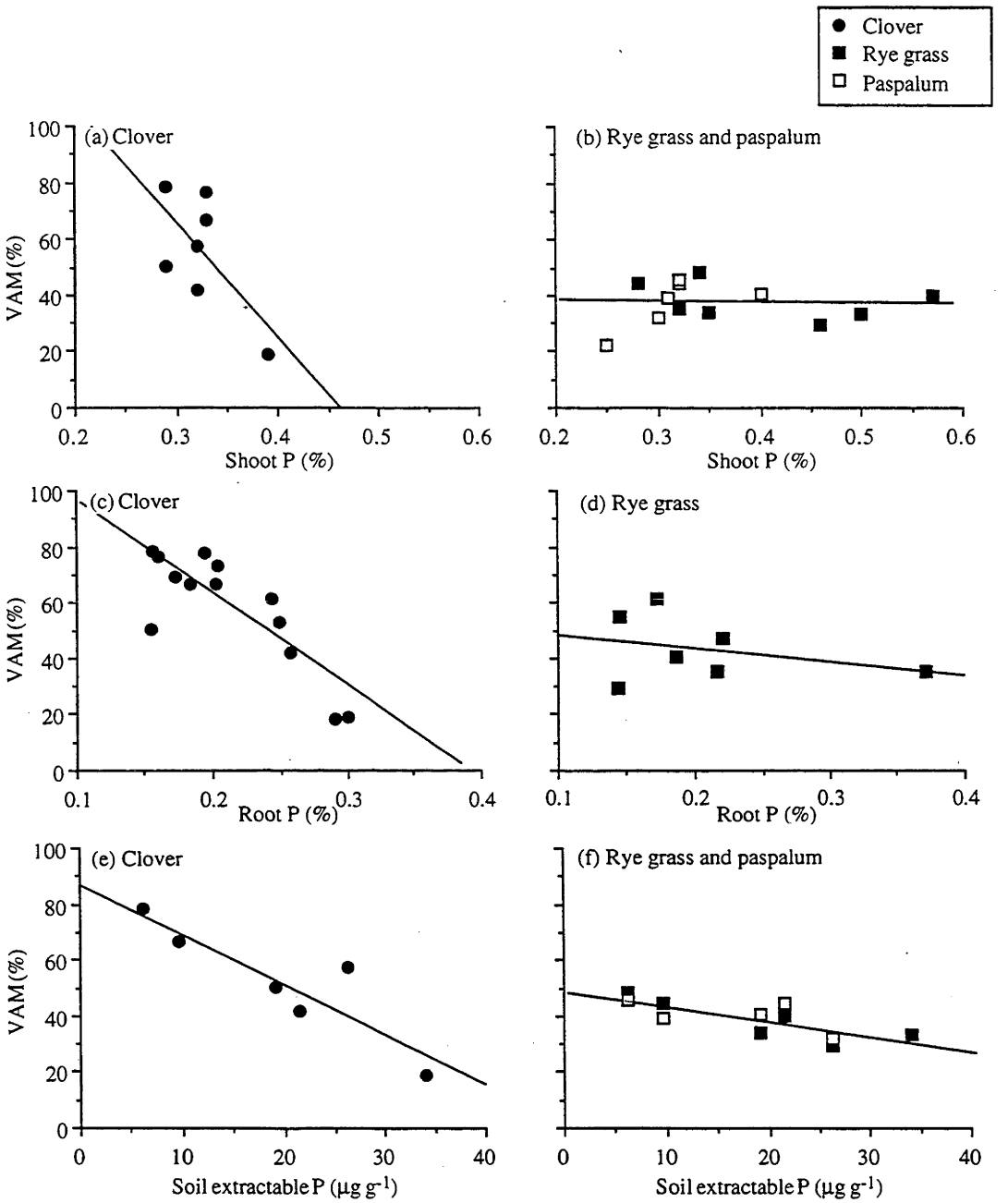


Figure 10.7. Simple regressions between the percentage of root length colonised by VAM fungi and a) clover shoot P (r^2=0.43), b) rye grass and paspalum shoot P (r^2=0.00), c) clover root P (r^2=0.68), d) rye grass root P (r^2=0.11), e) Olsen soil extractable P (clover) (r^2=0.81) and f) Olsen soil extractable P (rye grass and paspalum) (r^2=0.62). VAM colonisation levels, shoot P and soil extractable P are means from 15-20 sites in each paddock, while root P are from samples bulked at the paddock level. All data from January 1994, except extractable P which was measured in March 1994 (Small *et al.* 1994a) and some of the data in c) and all data in d) which was from October 1993.

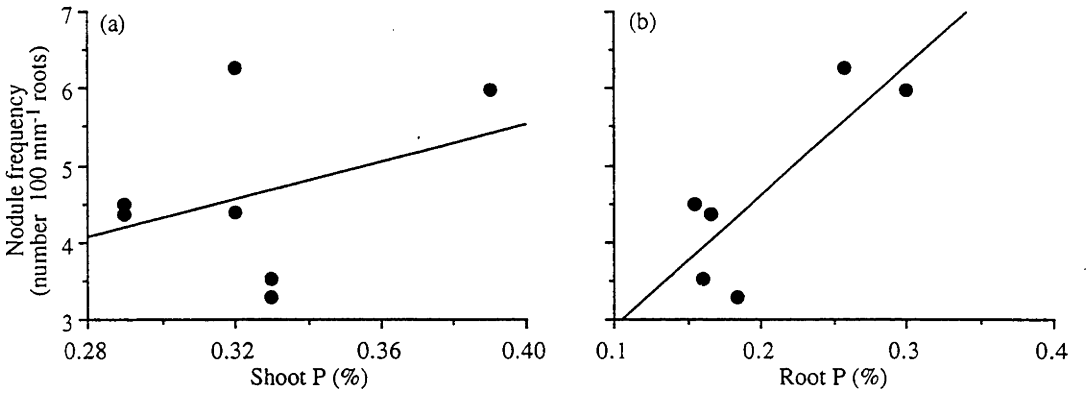


Figure 10.8. Simple regressions between the frequency of *Rhizobium* nodules (number 100 mm^{-1} of clover roots) and a) shoot P (r^2=0.13) and b) root P (r^2=0.69). Nodule data and shoot P are the means from 15-20 sites sampled in a paddock and root P are from samples bulked at the paddock level. Data from January 1994.

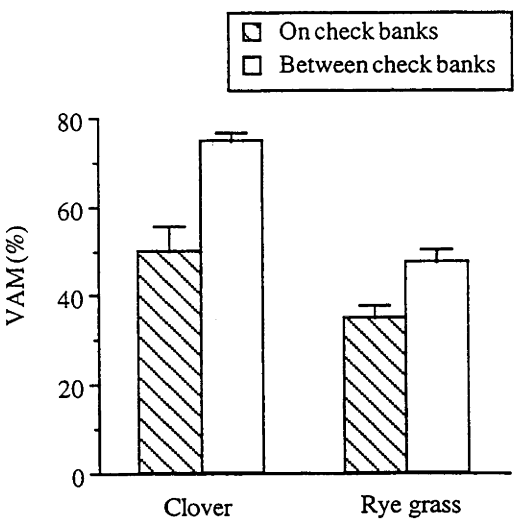


Figure 10.9. Percentage of clover and rye grass root length colonised by VAM fungi in pasture on irrigation check banks and in adjacent irrigated pasture on a biodynamic dairy farm; mean ± s.e.m., n=15.

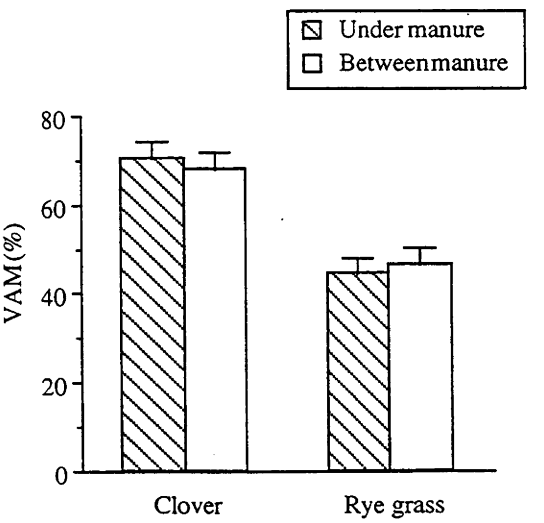


Figure 10.10. Percentage of clover and rye grass root length colonised by VAM fungi in patches of pasture where manure had been deposited four weeks previously and in adjacent unmanured pasture on a biodynamic dairy farm; mean ± s.e.m., n=15.

The effects of check banks and manure deposits on VAM fungi are shown in Figures 10.9 and 10.10. Samples taken on the check banks had around 33% less VAM colonisation than samples taken between check banks. Addition of manure four weeks previously had no effect on VAM colonisation.

(iv) Comparison of Relationships Between Farm Management Strategies

A stepwise regression examined whether the relationships between the measured variables were the same under the three farm management strategies (§3.9). The first set of analyses (Table 10.8) tested whether the two types of alternative farms, organic and biodynamic, were behaving in the same manner. Addition of management strategy only had a substantial effect in the pasture P versus log soil extractable P model. Addition of location greatly improved the fit of the two models where soil extractable P was added first. In the second set of analyses (Table 10.9), farm management strategy was divided into conventional and alternative. In all models, the addition of location resulted in the model explaining an additional 10% of the variation in the data. The addition of farm management strategy again markedly increased the fit only in the model of pasture P versus log soil extractable P.

Table 10.8. Stepwise regression analyses investigating whether location (Goulburn Valley, Murray River Valley, New South Wales, Gippsland, Tasmania, South Australia) or farm management strategy (biodynamic (BD), organic (O)) were effecting the VAM (%), other than through their influence on Olsen soil extractable P or pasture P. Farm averages from the 10 alternative NE Victorian farms and nine alternative farms located in other regions of SE Australia were used. The continuous variable was added first and location and farm management strategy were then added and the cumulative adjusted r^2, and the change in r^2, both recorded.

Dependent variable	Predictor variables	Adjusted r^2	Change in r^2
Clover VAM	log soil extractable P	0.42	+ 42
	+ location	0.66	+ 24
	+ *management strategy (BD/0)*	*0.67*	+ *1*
Clover VAM	pasture P	0.75	+ 75
	+ location	0.78	+ 3
	+ *management strategy (BD/0)*	*0.76*	- *2*
Pasture P	log soil extractable P	0.65	+ 65
	+ location	0.78	+ 13
	+ *management strategy (BD/0)*	*0.89*	+ *11*

Table 10.9. Stepwise regression analyses investigating whether location (Goulburn Valley, Murray River Valley, New South Wales, Gippsland, Tasmania, South Australia) or farm management strategy (conventional (C), alternative (A)) were effecting the VAM (%), other than through their influence on Olsen soil extractable P or pasture P. Farm averages from the 20 NE Victorian farms and 18 farms located in other regions of SE Australia were used. The continuous variable was added first and location and farm management strategy were then added and the cumulative adjusted r^2, and the change in r^2, both recorded.

Dependent variable	Predictor variables	Adjusted r^2	Change in r^2
Clover VAM	log soil extractable P	0.41	+ 41
	+ location	0.47	+ 6
	+ *management strategy (C/A)*	*0.51*	*+ 4*
Clover VAM	pasture P	0.36	+ 36
	+ location	0.48	+ 12
	+ *management strategy (C/A)*	*0.47*	*- 1*
Pasture P	soil extractable P	0.60	+ 60
	+ location	0.74	+ 14
	+ *management strategy (C/A)*	*0.82*	*+ 8*

10.3.b. Changes in VAM Colonisation over Time

To assess whether there were regular seasonal changes in VAM (%), colonisation in clover and rye grass was examined four times during the 10 months from March 1993 to January 1994. A further two samplings in November 1994 and November 1996 were made to assess whether the 1993 VAM colonisation levels remained stable over a longer period of time.

VAM colonisation levels on most farms varied by around 30% during 1993 (Figs. 10.11 and 10.12). This variation did not follow the same pattern on each farm. In particular, in Pair A there was a lot of variation on the biodynamic farm. However, when trends from 1993 to 1996 are viewed, VAM levels were relatively stable, or slightly increasing or decreasing on most farms. For instance, colonisation on the conventional farm in Pair B decreased over time, eventually being < 10% for both the clover and the grasses and colonisation on the conventional farm in Pair C also decreased steadily over time.

VAM colonisation in the paddock on the conventional farm in Pair A which had not received P fertiliser for 10 years was high in clover, similar to the neighbouring biodynamic paddock (Fig. 10.11.a). While for rye grass, VAM colonisation was intermediate to the conventional and biodynamic paddocks.

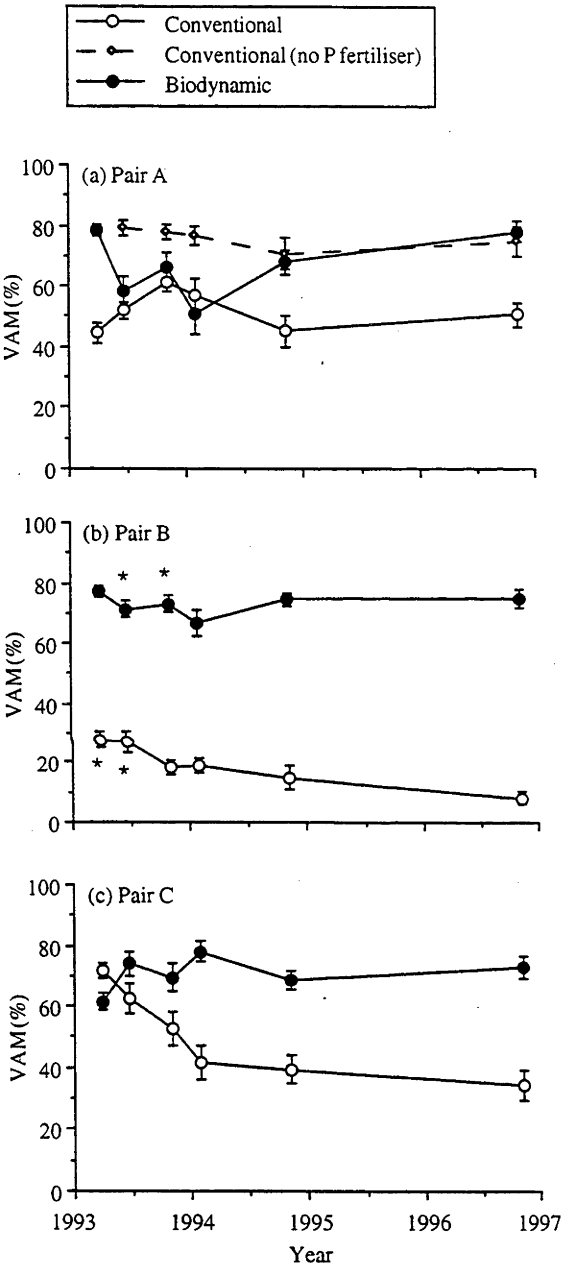


Figure 10.11. Percentage of clover root length colonised by VAM fungi on three pairs of conventional/biodynamic dairy farms in NE Victoria from March 1993 to November 1996; mean ± s.e.m., n=15-20. * indicates a different paddock was sampled.

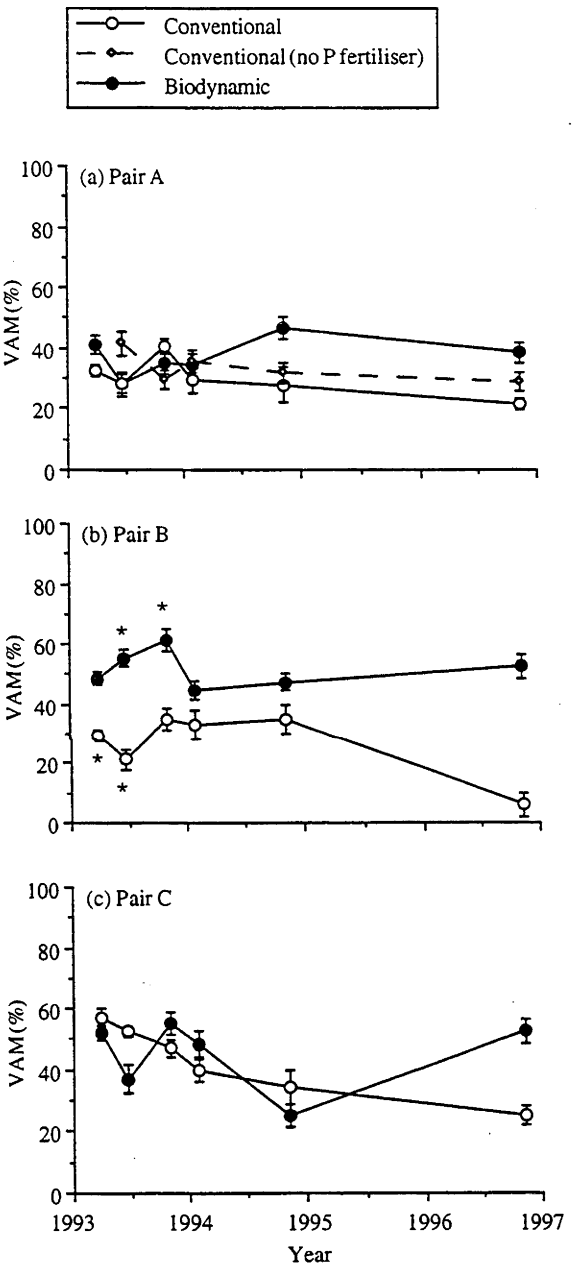


Figure 10.12. Percentage of rye grass root length colonised by VAM fungi on three pairs of conventional/biodynamic dairy farms in NE Victoria from March 1993 to November 1996; mean ± s.e.m., n=15-20. * indicates a different paddock was sampled.

Table 10.10 presents soil extractable P at five sampling dates between October 1991 and November 1996. On Pair A, extractable P remained relatively constant. On the conventional farms in Pairs B and C, extractable P increased after March 1993, and this was reflected in a drop in VAM colonisation levels (Fig. 10.11.b). The biodynamic farms in Pairs B and C also had a increase in extractable P, but this was not reflected in VAM colonisation levels. Note that the different methods of collection of the soil extractable P data means that interpretations of the trends in Table 10.10 must be made with caution.

Table 10.10. Trends in Olsen soil extractable P from October 1991 to November 1996 on three pairs of conventional (Con.) and biodynamic (BD) farms. Data from 1991, 1993 and 1994 were provided by Small and are the averages of 20 samples to 100 mm from each of two paddocks on each farm. Data from 1994 and 1996 are the averages from 15 samples to approximately 100 mm depth in one paddock on each farm which were bulked and one subsample analysed.

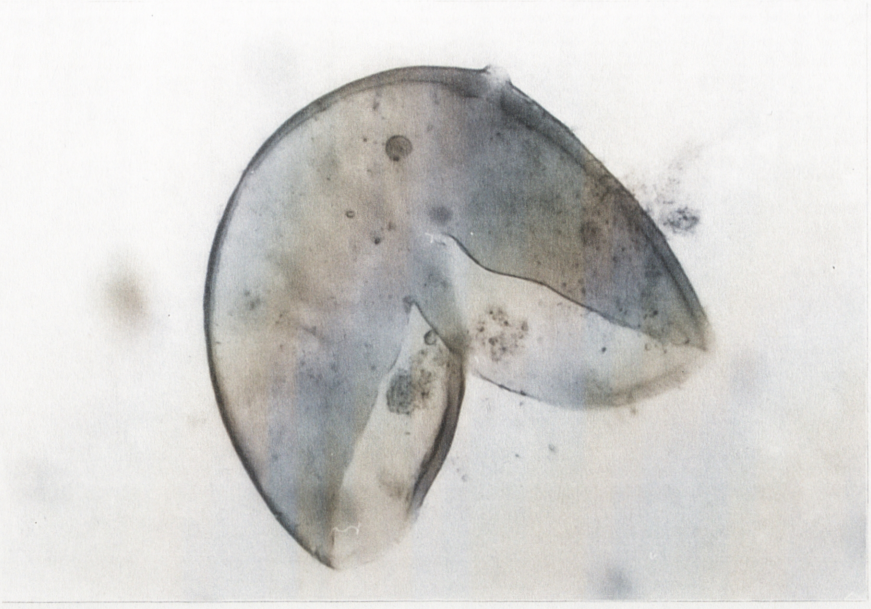
	Pair A		Pair B		Pair C	
	Con.	BD	Con.	BD	Con.	BD
October 1991	40	14	28	6	26	11
March 1993	27	10	18	4	24	15
March 1994	26	19	34	10	22	6
November 1994	27	8	74	32	62	26
November 1996	40	19	80	43	47	21

10.3.c VAM Spores

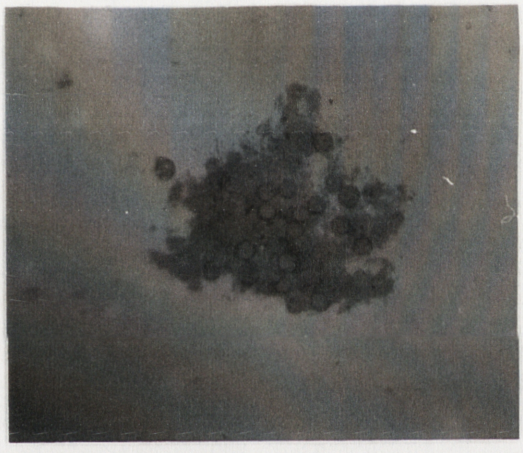
Six types of VAM spores were distinguished. These were 'large brown', 'small brown', 'medium-sized dark', 'medium-sized clear', small spores in 'clumps' and 'white oily'. The large brown were easy to distinguish due to their size (Plate 10.1.a). The small spores, which occurred in clumps of up to 30 spores, were distinctive and tended to be associated with organic matter (Plate 10.1.b). The white oil-filled spores (Plate 10.1.c) occurred infrequently. The other three types, not shown in the plates, were not as distinctive and classification was, at times, rather arbitrary. Identification of these spores was not attempted, however the large brown and small clumped appeared to belong to the genus *Glomus*, with the large brown possibly being *Glomus mosseae*, which has been recorded in this area by Hayman and Stovold (1979). All types of spores were present on all farms.

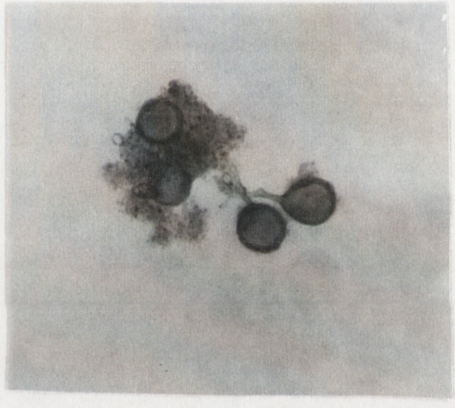
The spore data was analysed using two techniques. The first used principle components analysis to examine the co-occurrence of species (Fig. 10.13). The large brown spores tended to occur irrespective of the other species present. The medium-sized clear spores, small brown spores and white oily spores tended to occur together, whilst the clumped spores and medium-sized dark spores were less tightly associated with this group.

(a)



(b)





(c)

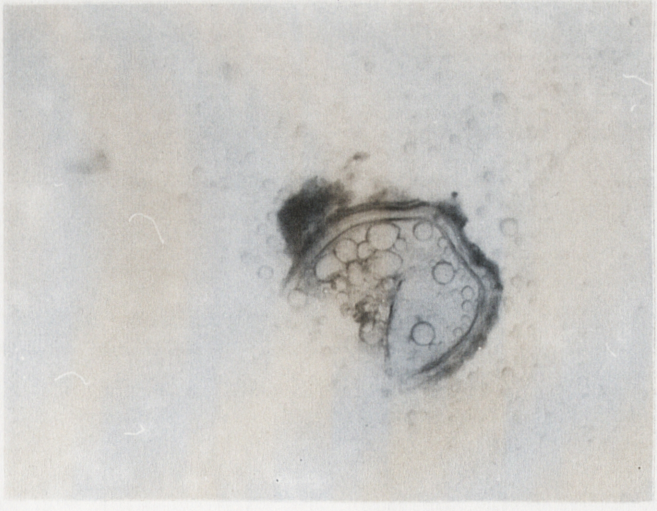


Plate 10.1. The three most distinguishable spore types found in the dairy farm pastures a) the large brown spore (x 100), b) the small clumped spore (x 50 and x 100) and c) the white oily spore (x 100).

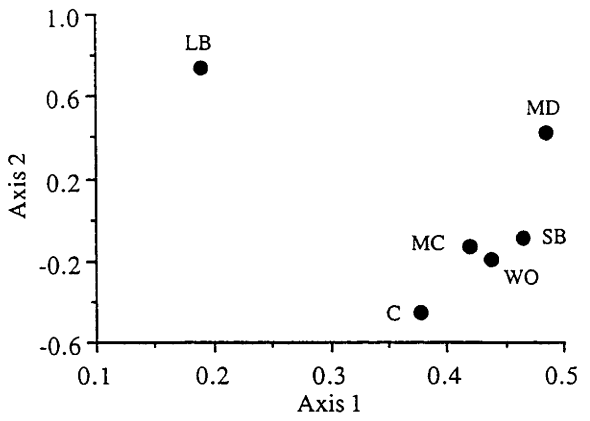


Figure 10.13. Co-occurance of VAM spore types. Axis 1 vs Axis 2 from a principle components analysis on a correlation matrix of the log spore abundance data; 55% of the variation in the data was explained by the first two axes. Data met the normality assumptions of the analysis. Spore types were large brown (LB), small brown (SB), medium-sized dark (MD), medium-sized clear (MC), small in clumps (C) and white oily (WO).

The influence of farm management strategy and location on spore abundance were examined through two sets of ANOVAs. In the first set, farm management strategy and location (farm pair) were fitted for each spore type (Table 10.11, Model 1). Management strategy had no effect for any of the spores, while location influenced only the small brown and medium-sized dark spores, both of which tended to be more abundant in Pairs B and C (Fig. 10.14). In the second set (Model 2), farm management strategy and paddock nested within the management strategy were fitted. Paddock was nested as there were not enough degrees of freedom to fit it alone, thus the models examined whether there were differences between the paddocks within each management strategy. Results were similar to those of Model 1.

Table 10.11. Summary of the significant predictor variables and their level of significance when abundance of each type of spore (number $gram^{-1}$ of dry soil) were fitted with management strategy (conventional, biodynamic) and either location (Pair A, Pair B, Pair C) or paddock nested within management strategy. There were not enough degrees of freedom to fit an interaction term. The conventional paddock which had not received P fertiliser for 10 years was excluded from these analyses.

	Model 1		Model 2	
	Management	Location	Management	Paddock [strategy]
Log large brown	-	-	-	-
Log small brown	-	0.009	0.03	<0.0000
Log medium dark	-	0.03	-	-
Log medium clear	-	-	-	-
Log clumps	-	-	-	0.03
Log white oily	-	-	-	-

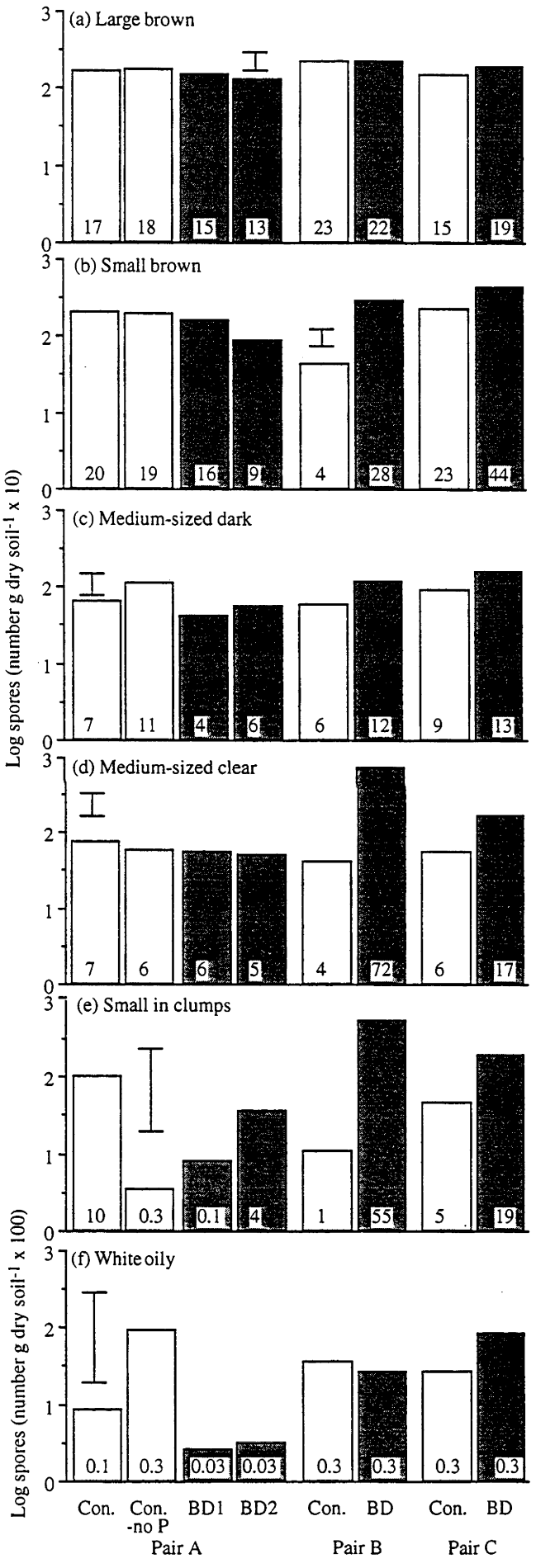


Figure 10.14. Estimated paddock means and LSD at p=0.05 from ANOVAs of log spore abundance in eight paddocks from three biodynamic (BD)/conventional (Con.) farm pairs. Two additional paddocks were sampled in Pair A; a paddock where no superphosphate had been applied for 10 years on the conventional farm (Con.-no P) and a second paddock on the biodynamic farm (BD2). Untransformed values are included at the base of each column. Values were multiplied by 10 (a-e) or 100 (f) to remove negative values. To allow inclusion of all paddocks, the model used to generate the means included only paddock, however the trends shown by the models in Table 10.11 are also apparent in these graphs.

The abundance of each spore type in each paddock is shown in Figure 10.14. The large brown spores occurred at a constant frequency in all paddocks. The small brown spores varied in distribution between paddocks and tended to be more abundant on the biodynamic farms. The medium-sized dark spores occurred at a fairly constant level on all farms. The medium-sized clear spores were more common on the biodynamic farms in Pairs B and C. The clumped spores were more abundant on the biodynamic farms in Pairs B and C, but were least abundant in the conventional paddock which had received no P fertiliser. The white oily spores were uncommon in all paddocks.

The two paddocks sampled on the biodynamic farm in Pair A had very similar numbers of all spore types, suggesting that variation is at the farm level, not the paddock level. The conventional paddock which had not received P fertiliser for 10 years also had very similar numbers of spores to its fertilised neighbour, with the exception of the small clumped spores. Overall, the biodynamic farms in Pairs B and C tended to have a greater number of spores than their conventional neighbour, but this trend was not apparent for Pair A.

10.4. Discussion

10.4.a. Soil and Pasture Nutrient Concentrations

Both soil extractable P and pasture P were significantly affected by farm management strategy, being higher on the conventional farms. This reflects the application of soluble P fertilisers on the conventional farms; on average 27 kg ha^{-1} $year^{-1}$ of P. Soil total N and pasture N were not affected by farm management strategy, even though the conventional farmers added more N fertiliser (Tables 9.2 and 9.3). Presumably, this was due to N entering the soil via biological fixation (see §10.4.d). Soil total P and N and pH differed significantly between locations, probably primarily due to differing underlying soil types.

Soil extractable P was measured by Small by the methods of Olsen *et al.* (1954) and Colwell (1963). These two measures were strongly linearly correlated, with the Colwell method consistently extracting four times more P. Both these tests use sodium bicarbonate as the extractant, with the Colwell method involving a wider solution/soil ratio and longer shaking time (Holford 1997). Thus for predicting plant growth responses, the two tests should be interchangeable for the soils covered in this project; although as it provides a broader range of values, the Colwell method may be preferable.

Nutrient concentrations were measured for the individual pasture species on the six time series farms. Clover from all farms had shoot P >0.25% and shoot N >3.3%; concentrations adequate for normal growth (Reuter and Robinson 1986; Weir and

Cresswell 1994). Shoot P in rye grass on all farms was >0.25%, indicating P was not limiting growth (Weir and Cresswell 1994). However, rye grass on all farms had shoot N below that necessary for normal growth (<3.5%) and rye grass on three of the farms was deficient in N (<2.0%) (Reuter and Robinson 1986; Weir and Cresswell 1994). Paspalum in all paddocks, except the conventional paddock which had not received P fertiliser, was not being limited by P (Reuter and Robinson 1986). No literature was found on normal paspalum shoot N.

Overall, shoot P tended to be lower on the biodynamic farms, but did not appear to be low enough to consistently limit plant growth. However, McDonald *et al.* (1994) found P concentrations in blood of cows in spring — when cows are at peak milk production — to be lower for biodynamic cows than conventional cows and suggested that P was limiting milk production on the biodynamic farms. In rye grass on all farms, shoot N was low enough to limit growth. As 40-50% of pasture biomass consisted of rye grass (Small *et al.* 1994a), N was the nutrient most limiting for pasture growth on the dairy farms (see also §11.1.d.iv).

10.4.b.Factors Influencing VAM Colonisation

(i) Farm Management Strategy and Farm Location

The level of VAM colonisation was significantly influenced by farm management strategy, irrespective of location, being consistently higher on the alternative farms. Two exceptions, where the conventional farm in a pair had a substantially higher level of colonisation, were located in northern NSW at Rollands Plains and Comboyne. The low VAM colonisation on the alternative farms in these two pairs corresponded with higher soil extractable P and pasture P than on the conventional farms. No additional background information was available on these farms, however the higher P concentrations on the alternative farms presumably reflect fertiliser additions before their conversion from conventional to alternative management.

(ii) Soil Factors, Biological Factors and Farm Management Practices

VAM colonisation levels were negatively correlated with soil extractable P and pasture P, with root P having a slightly stronger correlation with VAM colonisation than shoot P. Strong negative correlations between VAM colonisation and root P have also been found by Graham *et al.* (1981) and Lu *et al.* (1994). Lu *et al.* (1994) suggested that it is the P concentration in the portion of the root system being colonised, rather than the P status of the plant in general, which regulates VAM colonisation levels. The results in Figure 10.7 support this hypothesis (see §12.1.a.ii for further discussion).

However, while low pasture P always corresponded with VAM colonisation being high (around 80%), when pasture P reached 0.4%, VAM colonisation was quite variable (30-70%; Fig. 10.3.b). All dairy farms which had high pasture P and high

VAM colonisation were conventional (Fig. 10.3.b). Pasture P is determined by the underlying concentration of soil available P, as well as the most recent addition of P fertiliser. Aziz *et al.* (1991) found that adding a high level of P to soil did not reduce colonisation in plants which were already well colonised by VAM fungi. Thus high pasture P may exist with high VAM colonisation after a large addition of fertiliser has increased pasture P, but before the pasture has produced large quantities of new roots with lower VAM colonisation. Thus high VAM and high shoot P could occur together only on conventional farms and only when fertiliser additions were large and infrequent, as frequent additions of P would result in an equilibrium between pasture P and VAM colonisation. Alternatively, the conventional dairies had VAM fungi adapted to higher P levels, however there was no indication that this was the case on these farms (see §11.1.d.ii and §12.3.c.ii).

Neither soil total N or pasture N correlated with VAM colonisation or VAM intensity. However, shoot N in rye grass was below that necessary for normal growth, indicating that the concentration of nutrients where growth begins to be limited is not the same for VAM fungi and the host plant.

The conventional paddock which had not received P fertiliser for 10 years provided an opportunity to directly test whether the lower levels of VAM colonisation on the conventional farms were due to P fertiliser additions. VAM colonisation in clover in this paddock was consistently higher than in the adjacent conventional paddock which had regularly received P fertiliser (Fig. 10.11.a and see §10.4.b.iv). This strongly suggests that the addition of P fertiliser is the primary cause of the lower VAM colonisation on the conventional farms and that other management differences between conventional and biodynamic farms were not having a significant influence (see §10.4.e).

The sampling of irrigation check banks and under cow faecal deposits was done in order to isolate some of the factors responsible for the variation in VAM colonisation within individual paddocks. Check banks are usually drier than the rest of the paddock — as they are not inundated during irrigation — and also may be more compacted than the surrounding pasture due to cattle standing on them during irrigation (E. Hardie, pers. comm.). Both these factors could be contributing toward the lower VAM colonisation on the check banks. Indeed, it was noted during the assessment of VAM colonisation levels that the roots of plants growing on the check banks were relatively small and shrivelled, suggesting water stress.

The levels of VAM colonisation in patches of pasture which had received cow faecal deposits four weeks previously were not greater than in adjacent unmanured pasture. Fresh manure should contain around 1.2% P and 2.9% N (Haynes and Williams 1993). Four weeks may be inadequate for established VAM colonisation to

respond to such nutrient inputs, either through the breakdown of fungal structures in existing roots or through a lower rate of fungal growth in new roots.

The intensity of VAM colonisation was measured on all samples from the single survey of 38 farms and was strongly correlated with VAM (%). In contrast to the results from the crops, presented in Chapter 5, soil total N did not effect VAM intensity. No root length measures were made on the dairy farms during this project. However, Cock (1991) examined root length on one conventional/biodynamic farm pair in the Murray River Valley in NE Victoria. Root length in the top 0.1-0.6 m of soil was 13-80% greater on the biodynamic farm. This suggests that if the length of roots colonised by VAM fungi had been measured, it would have magnified the differences found in the VAM (%) data.

(iii) Differences in VAM Colonisation Levels between Clover and Grasses

Clover was generally more highly colonised than the grasses (Table 10.3) and the correlations between VAM (%) and soil and pasture nutrient concentrations were much stronger for clover (Tables 10.5 and 10.7). These correlations were also steeper than for the grasses, with clover being more highly colonised at low P and colonisation more reduced by high P (Fig. 10.7). This issue is discussed further in Chapter 12.1.c. Thus, the differences between the conventional and alternative farms were greater for the clover than the grasses.

(iv) Dynamics of VAM Colonisation over Time

The changes in VAM colonisation over 3.5 years on six farms were shown in Figures 10.11. and 10.12. On Pair A, the increase in VAM colonisation on the conventional farm during 1993 may have resulted from no P fertiliser being applied between August 1992 and three weeks prior to sampling in November 1993. Soil extractable P was also lower during this period. The decrease and variability of the VAM colonisation in clover in the biodynamic paddock during 1993 probably resulted from the construction of a dam in this paddock which began in May 1993. At the June 1993 sampling the paddock had just missed an irrigation and in November 1993 the soil was very dry, while other paddocks had just been irrigated. The lower VAM colonisation on check banks and the results in Chapter 8, suggest that low soil water will decrease VAM colonisation. The return to a higher VAM colonisation level on the biodynamic farm corresponded to the completion of the dam and a return to normal irrigation practices. Soil extractable P did not increase during the period of lower VAM colonisation (Table 10.10). Note that this paddock was the outlier in Figure 10.7.c where, compared to the other paddocks, it had a lower VAM (%) than would be expected given the concentration of P in the roots.

On Pair B, VAM (%) varied slightly during 1993, especially in the grasses. In the long term, colonisation was relatively stable on the biodynamic farm, but tended to decrease on the conventional. This decrease corresponded with a doubling of the soil extractable P (Table 10.10). The lowest VAM colonisation levels recorded in pasture during this project occurred on this farm in November 1996. The increase in extractable P on the biodynamic farm from March 1994 to November 1994 (10 to 32 $\mu g\ g^{-1}$) did not effect VAM colonisation levels; maybe more time would be required for colonisation levels to drop significantly. The increase in extractable P concentrations is unexpected. Being biodynamic, the farmer would not have been applying fertiliser containing readily soluble P, although small quantities of rock phosphate (16 kg ha^{-1} of P) were added in 1994 and 1996.

On Pair C, VAM colonisation on the biodynamic farm was steady for the clover, but quite variable for the rye grass, while on the conventional farm, colonisation decreased consistently over time. Ownership of the conventional farm had changed from father to son in 1992 and it is possible that fertiliser applications had decreased or ceased during the last years of the father's management, allowing VAM levels to increase. The decrease in VAM colonisation after 1993 may, therefore, have resulted from the renewal of fertiliser applications, particularly the 40 kg ha^{-1} of P applied in December 1992. The increase in soil extractable P during 1994 supports this scenario (Table 10.10).

It is possible that seasonal fluctuations in pasture P, unrelated to changes in soil extractable P, were responsible for the variations in VAM colonisation levels observed during 1993 (Figs. 10.11 and 10.12). Stockdale (1983) found that the P concentration of irrigated pasture at Kyabram varied with season, being highest in winter/spring (0.40-0.45%) and lowest in summer, particularly February (0.20-0.30%). Based on these results, VAM colonisation would be expected to be highest at the January 1994 sampling, however, this was not an obvious trend in the data. Overall, there was no evidence of marked seasonal variations in VAM colonisation.

The time series sampling also assessed whether one-off surveys are adequate to examine relationships between VAM colonisation and variables such as soil extractable P. If large fluctuations occurred in VAM colonisation due to factors such as season, one-off surveys may be inadequate. However, while a certain amount of variability was present and not reflected in changes in soil extractable P, the results suggest that a single sampling will be adequate in such perennial pastures.

10.4.c. Abundance of VAM Spores

It has been suggested that VAM species composition may vary in response to P fertiliser addition (Johnson 1993). While some spore types were more common on the biodynamic farms in Pairs B and C (Fig. 10.14), over-all, VAM spore types did not

vary consistently with farm management strategy, in spite of many years of differing P applications. Consequently this line of research was not pursued. See section 12.3.c.ii for further discussion.

However, the spore sampling methods used were relatively rudimentary and the results should be viewed cautiously. Sampling every month for two years may be necessary to get an accurate estimate of the species diversity at any site in the field, as presence may vary greatly with season and other environmental factors (Gemma *et al.* 1989; Pringle and Bever 1996). Moreover, all the VAM species present in the field may not be detected by spore extraction (Clapp *et al.* 1995; Johnson 1993). Although conversely, identifying fungi from field samples may lead to overestimation of species diversity, due to the variation present in spore age and condition (Morton, pers. comm.). A more rigorous assessment of the VAM species present on the dairy farms should involve repeated sampling over two years and trapping procedures using both soil dilutions and chopped roots from the field (Watson and Millner 1996). Establishment of pure cultures and the assessment of the effects of individual species, or groups of species (Edathil *et al.* 1996), on plant growth could then be attempted.

10.4.d. *Rhizobium* Nodulation

The frequency of *Rhizobium* nodules on clover roots was higher on the conventional farms and appeared to be positively correlated with root P. Coventry *et al.* (1985) also found that addition of P fertiliser at a site in SE Australia (13 kg ha^{-1} of P) increased the number of nodules on each plant. Phosphorus may indirectly limit N fixation through its effects on growth of the host plant (Ledgard and Steele 1992). Low populations of *Rhizobium* have been found to correspond low soil pH (Coventry *et al.* 1985; Evans *et al.* 1990), however, pH did not vary greatly between the farms in this project (Table 10.1).

These results suggest that the biodynamic farms — through not applying P fertilisers — may have lower rates of biological N fixation than the conventional farms, although the biodynamic farms did not have lower shoot N (Table 10.7). It is possible that the lower number of nodules corresponded with larger nodules (Coventry *et al.* 1985).

10.4.e. Were the Conventional and Alternative Farms Functioning through Similar Biological Relationships?

This question was addressed through stepwise regressions. When only organic and biodynamic farms were examined, the addition of farm management strategy did not significantly increase the r^2 for the models predicting VAM colonisation. When management strategy was divided into conventional and alternative, management strategy only explained a significant amount of variation for the relationship between pasture P and soil extractable P. This may be due to the differing extractable P

concentrations on the biodynamic and conventional farms and the non-linear relationship between soil extractable P and pasture P (Fig. 10.6). On the alternative farms, soil extractable P was relatively low and therefore tended to be strongly correlated with pasture P. In contrast, on the conventional farms, pasture P was generally high (>0.4%) and therefore would not be as strongly correlated with soil extractable P .

Location had a significant effect in most models, explaining an additional 3-24% of variation. This, presumably, reflected different underlying soil characteristics and environmental conditions.

10.5. Dairy Farm Pasture Conclusions

- The level of VAM colonisation was strongly negatively correlated with soil extractable P and pasture P. Soil N and pasture N did not correlate with VAM colonisation levels.
- The intensity of VAM colonisation was strongly correlated with VAM (%).
- There was a strong negative correlation between VAM colonisation and root P.
- The negative relationship between VAM and P was consistent across the broad range of locations sampled.
- VAM colonisation was consistently higher on the alternative farms due to lower concentrations of P in soil and pastures.
- VAM colonisation was higher in clover than grasses and the negative relationship between VAM (%) and P was not as strong for the grasses.
- VAM colonisation was lower on the irrigation check banks, probably due to lower levels of soil moisture.
- VAM colonisation levels did not vary in a regular seasonal pattern and remained relatively constant over the 3.5 year study period.
- Soil extractable P measured by the Olsen and Colwell methods was strongly linearly correlated.
- Pasture P was strongly positively correlated with soil extractable P.
- The numbers and types of VAM spores varied between farms, but did not vary consistently with farm management strategy.
- The frequency of *Rhizobium* nodules was greater on the conventional farms and positively correlated with root P.
- There was no indication that there were large differences between the biological processes on the conventional and alternative farms.

Chapter Eleven

Glasshouse Trials Examining VAM Colonisation and Plant Growth in Soils from NE Victorian Dairy Farms

This chapter presents four glasshouse trials which used soil from NE Victorian dairy farms. As no measures of plant growth were made on the dairy farms in the field, the first trial assessed the plant growth potential in soil from three conventional/biodynamic farm pairs. The trial also compared the effects of adding soluble P and N on VAM colonisation and plant growth in the conventional and biodynamic soils. The remaining trials compared the growth of plants with and without colonisation by VAM fungi. They also examined factors which affect the applicability of glasshouse trials to field conditions: addition of soluble P; regular partial defoliation; interspecific competition; the species of VAM fungi present; and plant density (intraspecific competition).

11.1. Effect of P and N on VAM Colonisation Levels and Growth of Clover and Rye Grass in Conventional and Biodynamic Soils

11.1.a. Aims

The aims of the glasshouse trial were to answer the following questions.

- What is the VAM inoculum potential in the three conventional and biodynamic soils?
- Is VAM inoculum potential a useful indicator of VAM colonisation levels in the field?
- What is the plant growth potential in the three conventional and biodynamic soils?
    - Will this differ between the conventional and biodynamic soils in response to the degree of VAM dependence of the plant species?
    - Will this differ between plant species which are "indigenous" to the dairy farms and those which are "foreign"?
- What effects do additions of soluble P and N have on plant growth and VAM colonisation levels?
    - Will VAM fungi indigenous to conventional and biodynamic soils respond in the same manner to nutrient additions?
    - Will plants grown in conventional and biodynamic soil respond in the same manner to nutrient additions?
- Do the internal concentrations of P and N in plants control the levels of VAM colonisation and *Rhizobium* nodulation?

11.1.b. Methods

Soil to 100 mm depth was collected in October 1994 from 20 sites in one paddock on three conventional/biodynamic dairy farm pairs in NE Victoria. At this time the paddocks were also sampled as part of a time series of measurements (§10.3.b). Farm pairs A, B and C denote the same farms as in Chapter 10. Soil from each farm was passed through a 10 mm sieve and bulked. Extractable P (Olsen *et al.* 1954) and total N were assessed on a subsample of each bulked soil by Wesfarmers CSBP (Perth, Western Australia; §3.6). Five factors were included in the experiment.

1) *Location*. Farm pair A, B, or C.

2) *Farm Management Strategy*. Conventional or biodynamic.

3) *Plant Species*. Five plant species were grown: wheat (*Triticum aestivum* L.) cv. Banks, sunflower (*Helianthos annuus* L.), canola (*Brassica napus* L.), white clover (*Trifolium repens* L.) cv. Kopo and perennial rye grass (*Lolium perenne* L.) cv. Yatsynl. The clover and rye grass cultivars were those recommended for the Kyabram district in

Plate 11.1. The glasshouse trial presented in section 11.1.

Plate 11.2. The glasshouse trial presented in section 11.2. Plants in the foreground had just been defoliated to 30 mm height. Note that some pots contain three species growing together (polyculture) while others contain only one species (monoculture).

NE Victoria by the Department of Agriculture. The degree of dependence on VAM fungi was expected to increase from the non-mycorrhizal canola, through rye grass, wheat and sunflower, to clover (§1.3.d).

4) *Phosphorus*. The four P treatments involved P added to the soil surface at planting and after 3 weeks as a solution of $NaH_2PO_4.2H_2O$ at 0, 10, 50 and 200 mg of P kg^{-1} of soil.

5) *Nitrogen*. The four N treatments involved N added to the soil surface at planting and after 3 weeks as a solution of NH_4NO_3 at 0, 9, 45 and 180 mg of N kg^{-1} of soil. The ratio of N to P was chosen to be similar to that of diammonium phosphate, a fertiliser applied on some of the conventional dairy farms.

The trial was not fully crossed. Nutrient treatment combinations (+) were applied to clover and rye grass as shown below, while the wheat, sunflowers and canola were grown without added nutrients.

P (mg kg^{-1} of soil)	0	9	45	180
200				+
50			+	+
10		+	+	+
0	+	+	+	+

N (mg kg^{-1} of soil)

Standard 100 mm pots were filled with 200 g of soil. Seeds were germinated in a 1:1 vermiculite:perlite mix and transplanted into the pots, two plants pot^{-1}, at the two leaf stage in early December 1994. Glasshouse temperatures ranged from 15-35°C and pots were weeded and watered by hand each day (§3.7). No basal nutrients were applied. Light levels were about 70% of daylight (up to 1500 µmol m^{-2} s^{-1}). Treatments were replicated seven times, with each set of replicates forming a randomised block in the glasshouse. Each block consisted of three sub-blocks, one for each farm pair. Within each sub-block, pots receiving the same treatment — one containing biodynamic soil and one containing conventional soil — were placed next to each other (Plate 11.1). These pairs of pots were arranged randomly within the sub-block.

The trial was harvested after five weeks and shoot dry weight and VAM colonisation levels measured (§3.3 and §3.5). Root dry weight was also measured for clover (§3.4.b). The shoots of clover and rye grass from four treatments in Pair A — no

added nutrients, 50 mg kg^{-1} of P, 45 mg kg^{-1} of N and 50 mg kg^{-1} of P + 45 mg kg^{-1} of N — were analysed for P and N concentration (§3.3). Five sets of statistical analyses were performed using the statistical package JMP® (§3.9).

1) ANOVAs analysing growth and VAM colonisation of the five plant species in conventional and biodynamic soil when no P or N was applied. Parameters fitted were block (1-7), location (Farm pair A, B, C) and management strategy (conventional, biodynamic). Results are presented graphically.

2) ANOVAs on clover and rye grass to examine the effects of P addition when no N was applied. Parameters fitted were block (1-7), P level (0, 10, 50, 200), location (Farm pair A, B, C) and management strategy (conventional, biodynamic). A 'P x management strategy' interaction term was included to assess whether the response to P differed conventional and alternative soils.

3) As for 2) but examining the effects of N level (0, 9, 45, 180) when no P was applied.

4) ANOVAs on clover and rye grass to examine whether N and P additions interacted. To construct a fully crossed data set, the highest rates of P and N addition were excluded. Parameters fitted were block (1-7), location (Farm pair A, B, C), P (0, 10, 50), N (0, 9, 45), management strategy (conventional, alternative), N x P, P x management strategy, N x management strategy and N x P x management strategy.

5) ANCOVAs assessing relationships between shoot nutrient concentrations and both VAM (%) and frequency of *Rhizobium* nodules. Data from the two farms and four nutrient treatments which were available for this analysis were treated as one data set. Parameters fitted were block (1-7) and a number of continuous variables: VAM (%); the frequency of *Rhizobium* nodules; shoot P; and shoot N.

A number of complications emerged during the trial. The large number of randomised pots meant that monitoring of water in individual pots was impossible. However, as the soils initially differed in texture, some soils were continually wetter. As the plants grew rapidly and soon required frequent watering, nutrients may have leached from the pots; hence the second application of the nutrient solutions.

11.1.c. Results

Table 11.1 presents the concentrations of soil extractable P and total N in the six soils used in the trial. Extractable P was 2-3 times higher in the conventional soils and was lower on Pair A than on Pairs B and C. Total N was also higher in the conventional soils, particularly in Pair C.

Table 11.1. Extractable P (Olsen) and total N in conventional (Con.) and biodynamic (BD) soils from the three farm pairs used in the glasshouse trial.

	Pair A		Pair B		Pair C	
	Con.	BD	Con.	BD	Con.	BD
Extractable P ($\mu g\ g^{-1}$)	27	8	72	32	62	26
Total N ($\mu g\ g^{-1}$)	5700	4400	6800	4000	10 700	5400

Figure 11.1 presents the VAM colonisation levels for four plant species — the canola plants were uncolonised — grown without additional nutrients in the six soils, that is, the VAM inoculum potential for each soil. For the clover, VAM (%) was significantly higher in the biodynamic soils. For the other species, VAM (%) was higher in the biodynamic soil in Pair A, but was similar in Pairs B and C. Overall in the biodynamic soils, colonisation levels were similar in the clover, sunflower and wheat, but much lower in the rye grass. In the conventional soils, colonisation was highest in the sunflower and wheat, and lower in the clover and rye grass.

Figure 11.2 presents the shoot dry weights for the five plant species grown without added nutrients. For Pair A, plant growth was consistently greater in the conventional soil, particularly for the clover. In Pairs B and C there was generally no significant difference in plant growth between the conventional and biodynamic soils. Plants in soil from Pair C tended to have a greater shoot weight than plants in soil from Pairs A and B for the less VAM dependent wheat, rye grass and canola.

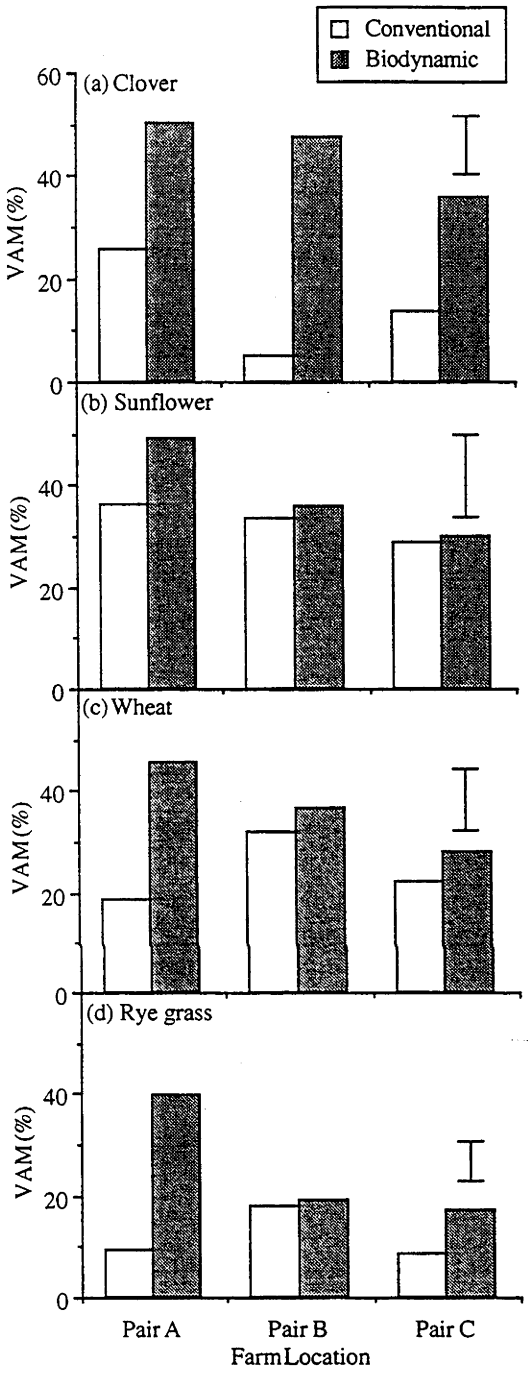


Figure 11.1. Percentage of root length colonised by VAM fungi in a) clover, b) sunflower, c) wheat and d) rye grass grown in soil with no additions of P or N from three conventional/biodynamic farm pairs; estimated means and LSD at p=0.05. Canola plants were uncolonised.

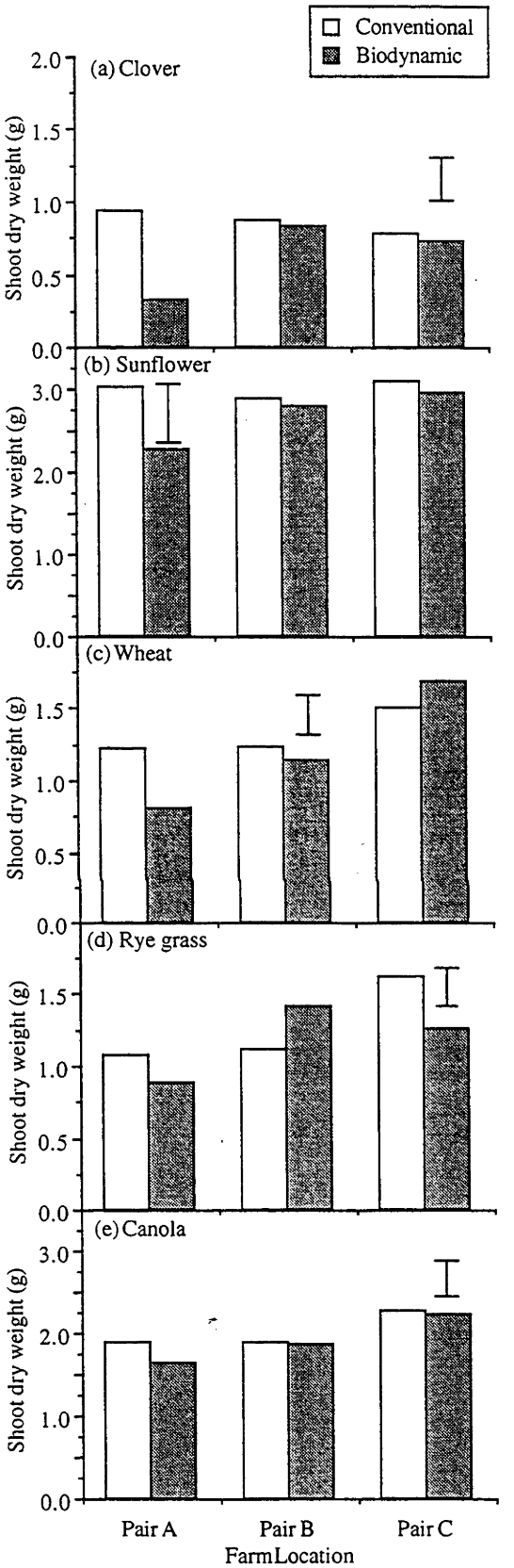


Figure 11.2. Shoot dry weight (g pot^{-1}) for a) clover, b) sunflower, c) wheat, d) rye grass and e) canola grown in soil with no additions of P or N from three conventional/ biodynamic farm pairs; estimated means and LSD at p=0.05.

The relationship between VAM (%) in the field and VAM (%) in the glasshouse trial is shown in Figure 11.3. Both clover and rye grass had colonisation levels around 10% lower in the glasshouse than in the field. For clover, VAM (%) in the field correlated strongly with the VAM inoculum potential; this was not the case for the rye grass. However, the slope of the regression line was identical for the two species.

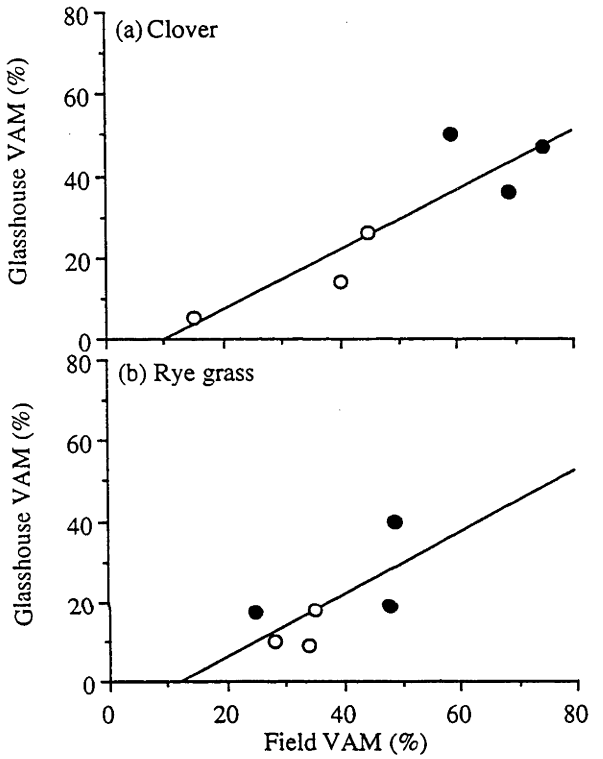


Figure 11.3. Simple regressions between the percentage of root length colonised by VAM fungi in the field — at the time when soil was collected to be used in the glasshouse trial — and the VAM colonisation level in the glasshouse trial (VAM inoculum potential) for a) clover (r^2=0.80) and b) rye grass (r^2=0.48). Farm management strategy is indicated. Points represent means from 15 sites in each paddock in the field and seven replicates in the glasshouse.

Table 11.2 presents the outcomes of analyses assessing both the effects of P addition on various aspects of clover growth and whether the conventional and biodynamic soils were responding to P addition in the same manner. The results are presented in Figure 11.4 as interactions between P addition and farm management strategy. Table 11.3 and Figure 11.5 contain the equivalent results for clover and N addition.

Table 11.2. Results from ANOVAs of clover grown under four levels of P addition (0, 10, 50, 200 mg kg^{-1}) in a glasshouse trial. Factors measured were the percentage of root length colonised by VAM fungi, shoot dry weight (g pot^{-1}), root dry weight (g pot^{-1}), the root-shoot ratio and *Rhizobium* nodules (nodules m^{-1} of roots). Parameters included are block (1-7), location (Farm pair A, B, C), P addition (0, 10, 50, 200) and the management strategy on the farm where the soil was collected (conventional, biodynamic). The interaction term, 'P x farm management strategy', assessed whether the response to P addition differed between conventional and biodynamic soil. There were no other significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	18.6	<0.0001	0.62	164
	block	1.3	0.3		
	location	27.9	<0.0001		
	P	35.6	<0.0001		
	management strategy	77.8	<0.0001		
	P x management strategy	10.2	<0.0001		
Shoot dry weight	full model	3.6	0.0008	0.14	164
	block	1.2	0.3		
	location	5.9	0.004		
	P	1.1	0.3		
	management strategy	19.5	<0.0001		
	P x management strategy	0.01	1.0		
Root dry weight	full model	1.5	0.1	0.06	163
	block	1.8	0.1		
	location	0.1	0.9		
	P	2.1	0.1		
	management strategy	4.3	0.04		
	P x management strategy	0.4	0.8		
Root-shoot ratio	full model	7.9	<0.0001	0.39	163
	block	8.0	<0.0001		
	location	16.1	<0.0001		
	P	1.9	0.5		
	management strategy	24.3	<0.0001		
	P x management strategy	2.0	0.1		
Rhizobium nodules	full model	2.7	0.001	0.15	164
	block	2.7	0.02		
	location	6.1	0.003		
	P	2.4	0.07		
	management strategy	3.6	0.06		
	P x management strategy	0.3	0.8		

Table 11.3. Results from ANOVAs of clover grown under four levels of N addition (0, 9, 45, 180 mg kg^{-1}) in a glasshouse trial. Factors measured were the percentage of root length colonised by VAM fungi, shoot dry weight (g pot^{-1}), root dry weight (g pot^{-1}), the root-shoot ratio and *Rhizobium* nodules (nodules m^{-1} of roots). Parameters included are block (1-7), location (Farm pair A, B, C), N addition (0, 9, 45, 180) and the management strategy on the farm where the soil was collected (conventional, biodynamic). The interaction term, 'N x farm management strategy', assessed whether the response to N addition differed between conventional and biodynamic soil. There were no other significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	18.1	<0.0001	0.60	166
	block	0.8	0.5		
	location	28.9	<0.0001		
	N	2.7	0.05		
	management strategy	197.0	<0.0001		
	N x management strategy	0.1	0.9		
Shoot dry weight	full model	3.8	<0.0001	0.20	166
	block	3.1	0.007		
	location	3.4	0.04		
	N	2.2	0.09		
	management strategy	23.8	<0.0001		
	N x management strategy	0.5	0.6		
Root dry weight	full model	1.3	0.2	0.04	166
	block	1.9	0.08		
	location	1.8	0.2		
	N	0.5	0.7		
	management strategy	2.1	0.2		
	N x management strategy	0.2	1.0		
Root-shoot ratio	full model	8.3	<0.0001	0.40	165
	block	6.2	<0.0001		
	location	18.8	<0.0001		
	N	0.8	0.4		
	management strategy	47.3	<0.0001		
	N x management strategy	0.3	0.8		
Rhizobium nodules	full model	4.7	<0.0001	0.25	165
	block	1.6	0.1		
	location	6.0	0.003		
	N	6.3	0.0005		
	management strategy	23.9	<0.0001		
	N x management strategy	2.8	0.05		

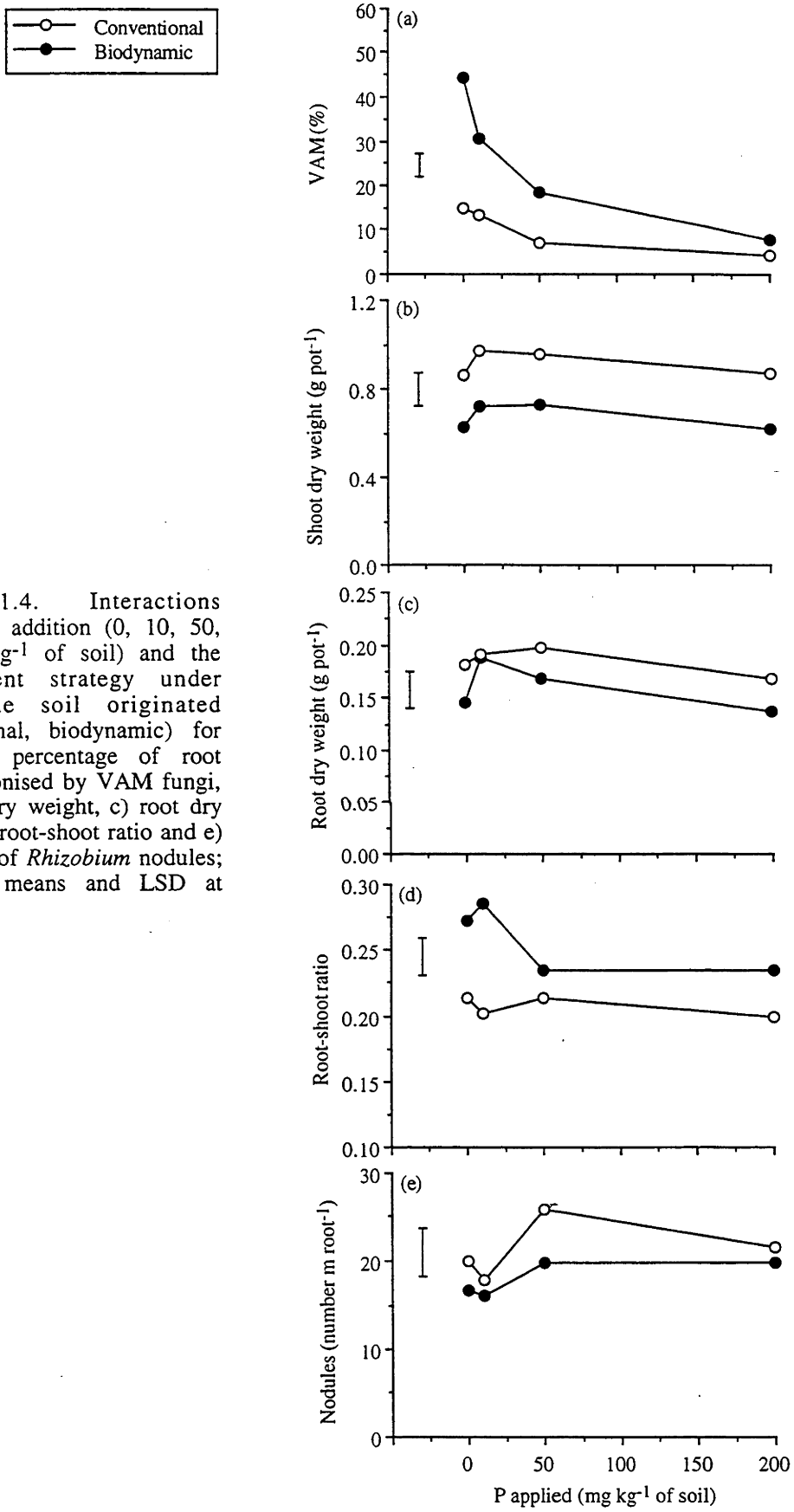


Figure 11.4. Interactions between P addition (0, 10, 50, 200 mg kg^{-1} of soil) and the management strategy under which the soil originated (conventional, biodynamic) for clover a) percentage of root length colonised by VAM fungi, b) shoot dry weight, c) root dry weight, d) root-shoot ratio and e) frequency of *Rhizobium* nodules; estimated means and LSD at p=0.05.

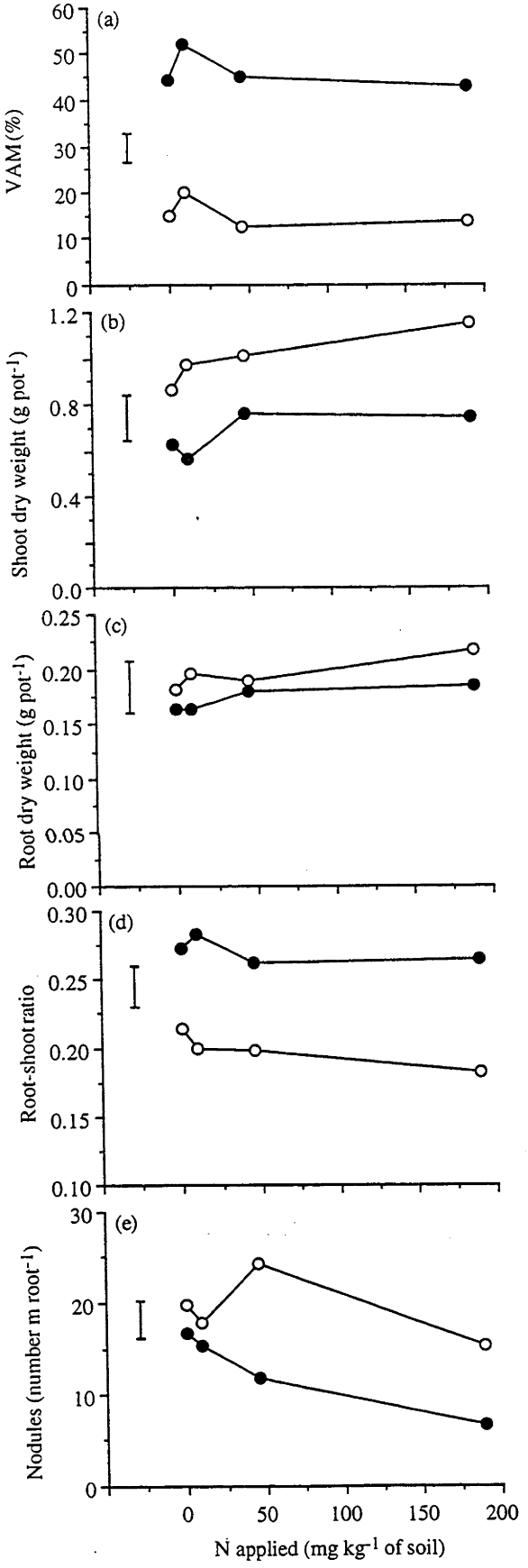


Figure 11.5. Interactions between N addition (0, 9, 45, 180 mg kg^{-1} of soil) and the management strategy under which the soil originated (conventional, biodynamic) for clover a) percentage of root length colonised by VAM fungi, b) shoot dry weight, c) root dry weight, d) root-shoot ratio and e) frequency of *Rhizobium* nodules; estimated means and LSD at p=0.05.

VAM colonisation of clover differed between soil from the three locations and was consistently significantly higher in the biodynamic soils. The addition of P reduced colonisation, an effect that was significantly more marked in the biodynamic soils. The addition of N caused a small increase in colonisation at the lowest level, but had no effect on colonisation at the higher two levels (Figs. 11.4.a and 11.5.a).

Clover shoot dry weight was higher in the conventional soils and was not significantly affected by P addition (Fig. 11.4.b). Phosphorus initially increased growth, but growth decreased again at the higher levels of P. Addition of N tended to increase shoot weights, although not significantly, particularly in the conventional soils (Fig 11.5.b). Root dry weight consistently tended to be higher in the conventional soils and, in a similar response to that of the shoots, P initially increased root weight, particularly in the biodynamic soils. Nitrogen addition tended to increase root weight. The variation in root weights was not well described by the models which, overall, were not significant.

The root-shoot ratio of clover differed significantly between plants grown in soils from the three locations and was significantly higher in the biodynamic soils. Neither addition of P or N had a consistent significant effect on the root-shoot ratio. However, in the biodynamic soils, the root-shoot ratio was significantly lowered by the two highest levels of P (Fig. 11.4.d).

The frequency of *Rhizobium* nodules varied with location and tended to be higher in the conventional soils, particularly at the two highest levels of N. In both soils, addition of P increased nodule frequency by approximately 10%. Addition of N consistently decreased nodule numbers in the biodynamic soils, while in the conventional soils, nodule numbers remained steady except for a large increase at the addition of the middle level of N (Figs. 11.4.e and 11.5.e).

Tables 11.4 and 11.5 and Figures 11.5 and 11.6 present the outcomes from analyses of the effects of P and N addition on shoot growth and VAM (%) in rye grass. VAM colonisation differed significantly between locations and was significantly higher on the biodynamic farms. Addition of P did not significantly consistently influence VAM colonisation, however, it did cause a decrease in colonisation in the biodynamic soils (Fig. 11.6.a). Addition of N did not significantly influence VAM colonisation. Shoot dry weight differed significantly between locations. Addition of P did not significantly influence shoot growth. At the highest two levels of P, plants in the biodynamic soil were heavier than those in the conventional. Addition of N significantly increased shoot growth. This was a stronger effect in the conventional soil, resulting in plants in the conventional soil being heavier than those in the biodynamic soil, particularly at the highest level of N addition (Fig. 11.7.b).

Table 11.4. Results from ANOVAs of rye grass grown under four levels of P addition (0, 10, 50, 200 mg kg^{-1}) in a glasshouse trial. Factors measured were the percentage of root length colonised by VAM fungi and shoot dry weight (g pot^{-1}). Parameters included are block (1-7), location (Farm pair A, B, C), P addition (0, 10, 50, 200) and the management strategy on the farm where the soil was collected (conventional, biodynamic). The interaction term, 'P x farm management strategy', assessed whether the response to P addition differed between conventional and biodynamic soil. There were no other significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	7.0	<0.0001	0.31	165
	block	5.5	<0.0001		
	location	12.3	<0.0001		
	P	0.9	0.5		
	management strategy	24.5	<0.0001		
	P x management strategy	1.6	0.2		
Shoot dry weight	full model	3.2	0.0002	0.17	164
	block	2.9	0.01		
	location	10.0	0.0001		
	P	0.9	0.5		
	management strategy	1.4	0.2		
	P x management strategy	2.1	0.1		

Table 11.5. Results from ANOVAs of rye grass grown under four levels of N addition (0, 9, 45, 180 mg kg^{-1}) in a glasshouse trial. Factors measured were the percentage of root length colonised by VAM fungi and shoot dry weight (g pot^{-1}). Parameters included are block (1-7), location (Farm pair A, B, C), N addition (0, 9, 45, 180) and the management strategy on the farm where the soil was collected (conventional, biodynamic). The interaction term, 'N x farm management strategy', assessed whether the response to N addition differed between conventional and biodynamic soil. There were no other significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM (%)	full model	9.5	<0.0001	0.43	168
	block	1.4	0.2		
	location	19.6	<0.0001		
	N	0.3	0.9		
	management strategy	88.5	<0.0001		
	N x management strategy	1.8	0.1		
Shoot dry weight	full model	12.0	<0.0001	0.50	166
	block	8.2	<0.0001		
	location	23.0	<0.0001		
	N	22.3	<0.0001		
	management strategy	10.7	0.001		
	N x management strategy	2.6	0.05		

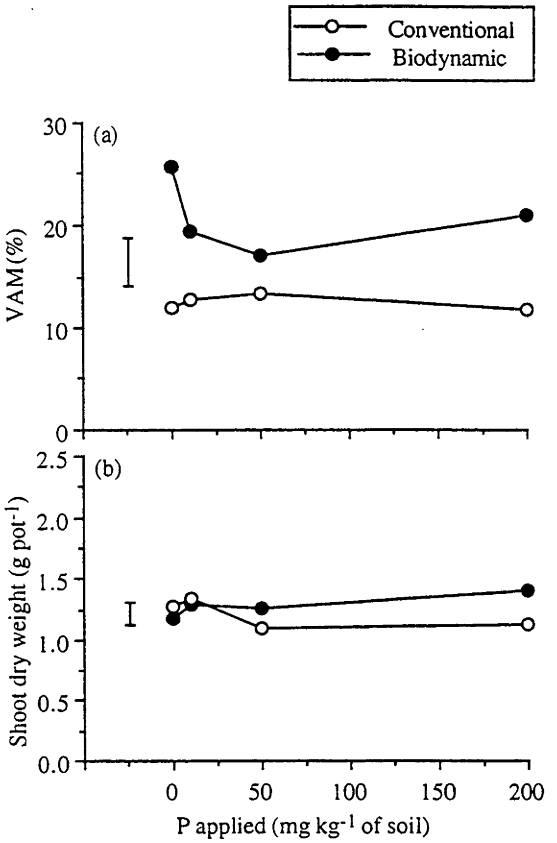


Figure 11.6. Interactions between P addition (0, 10, 50, 200 mg kg^{-1} of soil) and the management strategy under which the soil originated (conventional, biodynamic) for rye grass a) percentage of root length colonised by VAM fungi and b) shoot dry weight; estimated means and LSD at p=0.05

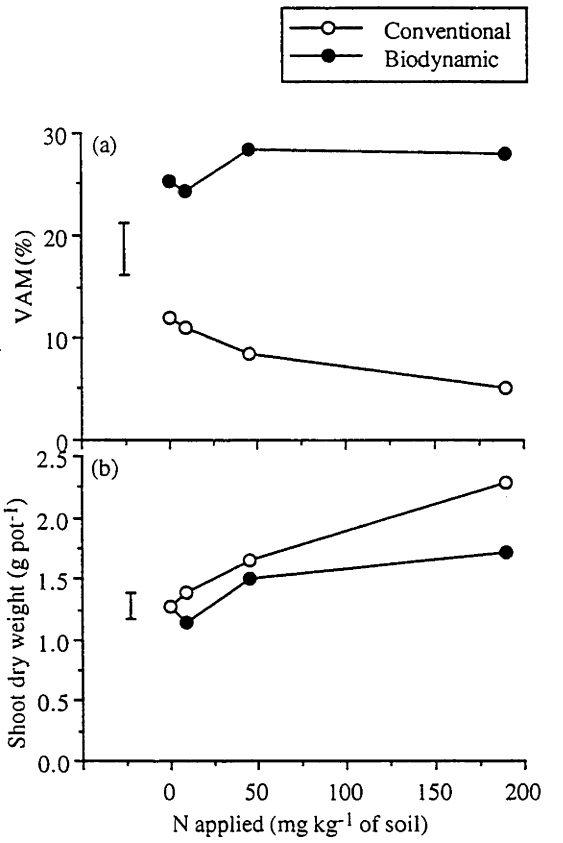


Figure 11.7. Interactions between N addition (0, 9, 45, 190 mg kg^{-1} of soil) and the management stratgey under which the soil originated (conventional, biodynamic) for rye grass a) percentage of root length colonised by VAM fungi and b) shoot dry weight; estimated means and LSD at p=0.05

Overall, the only instance where there was a strong, consistent, significant interaction between nutrient addition and the management strategy under which the soil originated was the effect of P addition on VAM colonisation in clover; the effects of N addition on rye grass shoots and nodulation in clover were significant at only $p<0.05$.

A model was fitted to all data, excluding the highest levels of P and N, to assess whether there was an interaction between P and N, or between P, N and farm management strategy. The factors examined for clover were VAM (%), shoot dry weight, root dry weight, root-shoot ratio, and the frequency of *Rhizobium* nodules, while VAM (%) and shoot dry weight were examined in rye grass. In all cases the two interaction terms were not significant at $p \leq 0.05$ and these results are not presented.

To more closely investigate the factors responsible for VAM colonisation levels and the frequency of *Rhizobium* nodules, shoot P and N were determined for plants from four nutrient treatments from Pair A (Table 11.6 and Fig. 11.8). VAM (%) in clover and rye grass was strongly negatively correlated with shoot P, although the relationship was stronger for clover than rye grass. Shoot N did not have a significant effect on VAM (%). The frequency of *Rhizobium* nodules was positively influenced by shoot P and negatively influenced by shoot N.

Table 11.6. Results from ANCOVAs of clover and rye grass grown in the glasshouse trial in soil from the conventional and biodynamic farms in Pair A under four nutrient treatments: no nutrient addition, P added at 50 mg kg^{-1} of soil, N added at 45 mg kg^{-1} of soil and P and N added together at these rates. Factors measured were the percentage of root length colonised by VAM fungi and *Rhizobium* nodules (nodules m^{-1} of root length). Parameters included are block (1-7) and shoot P and N concentration (%). There were no significant interaction terms.

Dependent variable	Predictor variable	Co-efficient	s.e.	F-ratio	Prob.	r^2	n
Clover VAM (%)	model	-	-	14.2	<0.0001	0.64	52
	block	-	-	2.1	0.08		
	shoot P %	-177.7	19.0	87.3	<0.0001		
Clover nodules	model	-	-	2.9	0.01	0.23	52
	block	-	-	2.5	0.04		
	shoot P %	41.5	16.0	6.7	0.01		
	shoot N %	-9.7	4.7	4.2	0.05		
Rye VAM (%)	model	-	-	3.6	0.004	0.25	55
	block	-	-	1.4	0.2		
	shoot P %	-83.0	18.8	19.4	0.0001		

Shoot P and N of the plants in Pair A which received no nutrient additions are presented in Table 11.7. Shoot P was significantly higher in plants grown in the conventional soil, while shoot N was similar in plants grown in the conventional and biodynamic soils.

Table 11.7. Concentrations of P and N in shoots of clover and rye grass grown in the glasshouse trial in soils from the conventional and biodynamic farms in Pair A with no nutrient additions; mean *(s.e.m.)*, n=7.

		Conventional	Biodynamic
Clover	Shoot P	0.36 *(0.03)*	0.24 *(0.02)*
	Shoot N	3.11 *(0.11)*	2.87 *(0.10)*
Rye grass	Shoot P	0.33 *(0.01)*	0.22 *(0.02)*
	Shoot N	1.95 *(0.12)*	2.14 *(0.12)*

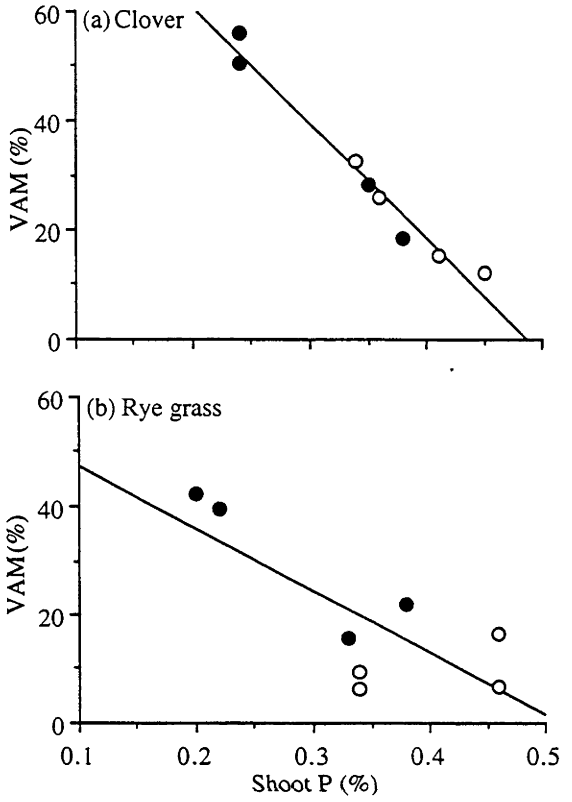


Figure 11.8. Simple regressions between a) percentage of root length colonised by VAM fungi and shoot P in clover (r^2=0.97) and b) percentage of root length colonised by VAM fungi and shoot P in rye grass (r^2=0.61). Each point represents the average of seven replicates from one of four treatments (no nutrient addition, P applied at 50 mg kg^{-1} of soil, N applied at 45 mg kg^{-1} of soil, and P and N applied together at these rates) in either conventional or biodynamic soil from Farm pair A. Farm management strategy is indicated.

11.1.d. Discussion

(i) The VAM Inoculum Potential of the Soils

VAM inoculum potential is routinely compared between field sites by growing bait plants in a glasshouse in soil collected from the field (Brundrett *et al.* 1996; Brundrett and Abbott 1994; Scheltema *et al.* 1987). A highly VAM dependent host plant, such as clover, is generally grown. In the present trial, clover grown in conventional soil was poorly colonised compared to clover grown in biodynamic soil and the VAM colonisation levels in the glasshouse and the field were strongly linearly correlated (Fig. 11.3.a). The consistently lower level of colonisation in the glasshouse was probably due to the plants having a higher growth rate than in the field and their younger age.

Thus differences in VAM (%) in treatments in glasshouse trials are likely to be similar, and in proportion, to those in the field. This strong correlation supports the use of glasshouse trials to compare plant growth potentials — presumably a function of soil nutrient concentrations and VAM colonisation levels — between field soils from various sites.

Colonisation levels in the other species in the glasshouse trial did not closely reflect those in the clover. Both the sunflowers, which are considered highly dependent on VAM fungi (Thompson 1987) and wheat and rye grass, which are poorly dependent (Chapter 7 and §11.2), did not show the large differences evident in the clover. This was particularly so for Pair B, where colonisation of clover was far higher in the biodynamic soil, but rye grass had a similar level of colonisation in the conventional and biodynamic soils. Thus, when assessing the VAM inoculum potential of field soils a number of host plant species should be used and — depending on the question being addressed — plants present at the field site should be included.

There was no indication that the plant species indigenous to the pasture system, white clover and rye grass, were more readily colonised than the foreign plants, sunflower and wheat. This supports the accepted theory that VAM fungi do not exhibit strong host plant specificity (Smith and Read 1997).

(ii) The Plant Growth Potential of the Soils

The potential of five species to grow in soil from three farm pairs was assessed. The biodynamic soil in Pair A produced consistently smaller plants than all the other soils, reflecting low extractable P (8 $\mu g\ g^{-1}$). This difference decreased as the VAM dependency of the plants declined, perhaps due to N beginning to limit plant growth as the plant requirement for P, relative to N, decreased and/or plants possessing alternative mechanisms for enhancing P uptake, such as fibrous root systems (Föhse *et al.* 1991; see discussion in §12.1.c). The wheat, rye grass and canola grew faster in the soil from Pair C, perhaps due to higher soil N (Table 11.1). For Pairs B and C there were no consistent differences in growth between the conventional and biodynamic soils.

Figure 11.2 and Table 11.1 indicated that soil extractable P became limiting for plant growth between 8 and 26 $\mu g\ g^{-1}$. This is consistent with the classification for Olsen P used by the State Chemistry Laboratories in Victoria, cited in Small *et al.* (1994a), where <12 $\mu g\ g^{-1}$ is considered low and 12-18 $\mu g\ g^{-1}$ marginal. Thus, of the 10 biodynamic dairy farms sampled in NE Victoria in March 1993 (§10.3.a), six had low and two had marginal concentrations of soil extractable P, compared with three conventional farms in each category. In the soils used in this trial, P would be expected to be limiting plant growth only in the biodynamic soil from Pair A.

(iii) The Effect of Nutrient Additions on VAM Fungi

Addition of P strongly reduced VAM colonisation in clover (Fig. 11.4.a), while VAM colonisation in rye grass did not show a significant response to P. The correlation between shoot P and VAM colonisation was much stronger in the glasshouse than in the field (Fig. 10.7.a), presumably reflecting the more uniform and optimum growth conditions in the glasshouse, as well as the even age of the plants and the absence of

grazing and diseases. Nitrogen addition did not significantly affect VAM colonisation (Tables 11.3 and 11.5) and shoot N did not correlate with VAM colonisation (Table 11.6); this is consistent with the field results (§10.4.b.ii).

There was a significant interaction between P and farm management strategy (Table 11.2), with VAM colonisation of clover in the biodynamic soils being more reduced at the first two levels of P than in the conventional soils. This could indicate that the VAM fungi in the conventional soils, after >15 years of applications of soluble P fertiliser, had adapted to be more tolerant to P; a conclusion consistent with the results of Jasper *et al.* (1979) and Johnson (1993). However, the contrasting levels of colonisation in the conventional and biodynamic soils when no P was applied, makes interpretation of results difficult. The non-linear relationship between soil and plant P concentrations (Fig. 10.6) means that the addition of P may have had relatively little influence on plant P in the conventional soils with their higher extractable P. Also, the relationship between the VAM colonisation and shoot P was identical for clover in conventional and biodynamic soils, suggesting that VAM fungi in conventional soil were not more tolerant of P (Fig. 11.8.a). However, it is still possible that the VAM fungi present in the conventional soils were more efficient at enhancing plant P uptake under high P conditions than the fungi in the biodynamic soils. This was not tested during this project. This issue is discussed further in section 12.3.c.ii.

(iv) The Effect of Nutrient Additions on Plant Growth

Clover shoot dry weight was increased by 15% at the lowest level of P addition in both conventional and biodynamic soil (Fig. 11.4.b). However, by the highest level of P addition, growth was decreasing, perhaps indicating that P concentrations were becoming toxic. A similar trend was evident for the clover roots. Phosphorus had no effect on growth of the rye grass. Thus, P was generally not limiting plant growth in these soils, with the possible exception of the biodynamic soil from Pair A (§11.1.d.ii).

Nitrogen increased clover and rye grass shoot dry weight, particularly in the conventional soil. At the highest level of N addition, plants in the biodynamic soil may have become limited by P. While no significant interactions were found between P and N, the highest levels of P and N were not included in the analysis, as they were not crossed. Nitrogen had a positive, but not significant, effect on clover root weight. Thus N appeared to be the major limiting nutrient in the dairy soils; a finding consistent with the field results (§10.4.a).

The root-shoot ratio in clover was not affected by P addition in the conventional soil, but was significantly reduced at the highest two levels of P addition in the biodynamic soils (Fig. 11.4.d). It was also consistently higher in the biodynamic soils. This seems to indicate that P was limiting clover growth in the biodynamic soils, making it strange that the shoot response to the increasing P additions was not larger.

The root-shoot ratio also showed a tendency to decrease as N increased (Fig. 11.5.d). This is the expected response to addition of a limiting nutrient (Wilson 1988b), although the ability of clover to biologically fix N may have resulted in the trend not being more marked.

Shoot nutrient concentrations were measured in four treatments in Pair A (Table 11.7). Clover shoot P was 0.22% in the biodynamic soil and 0.33% in the conventional soil. Phosphorus concentrations <0.23% are considered low (Weir and Cresswell 1994) and may affect plant growth. This is reflected in the poor growth of the biodynamic clover in Pair A compared to the other pairs (Fig. 11.2, and §11.1.d.ii). While the P concentration of the clover in the conventional soil (0.33%) was similar to that in the field in January 1994 (0.32%, Table 10.6), the biodynamic clover in the field had much higher shoot P (0.29%) than in the trial. Clover shoot N was low in the conventional soil (3.1%) and deficient in the biodynamic soil (2.8%; Weir and Cresswell 1994); both were lower than in the field samples taken in January 1994 (>3.4%).

The concentration of P in the rye grass (0.33%, 0.22%) in both soils was sufficient for normal growth (Weir and Cresswell 1994), but was considerably lower than the concentrations in the field in January 1994 (0.46%, 0.35%). Shoot N in the rye grass (2.0%, 2.1%) was significantly below that necessary for normal growth (≥3.5%; Weir and Cresswell 1994). This is consistent with the strong increase in rye grass growth when N was applied in the trial (Fig. 11.7.b). The concentrations were similar in the field in January 1994 (2.4%, 2.1%).

Thus, shoot nutrient concentrations in the glasshouse trial were often lower than in the field. This could be due to the small size of the pots in the glasshouse trial or the more ideal growth conditions in the glasshouse. Under field conditions — in a well established pasture sward — factors such as competition, water, light, disease and grazing will be limiting plant growth. As a result, nutrients may accumulate in the plant and concentrations of soil available nutrients which may limit growth in a glasshouse trial may not do so in the field.

(v) The Effect of Nutrient Additions on Rhizobium Nodules in Clover

Addition of P tended to increase nodule frequency (Fig. 11.4.e), while addition of N consistently decreased nodules on clover in the biodynamic soil. In the conventional soil, the second level of N inexplicably increased nodule numbers (Fig. 11.5.e). As in the field (Table 10.6), nodules occurred more frequently in plants grown in conventional soil. This was particularly the case in Pair A; 20.6±3.7 and 12.8±2.1 nodules m^{-1} of root length in the conventional and biodynamic soils respectively. When shoot nutrient concentrations were determined for four treatments in Pair A, nodule numbers had been increased by P addition and decreased by N addition (Table 11.6). Overall, it appears that the low soil extractible P on the biodynamic farms result

in less nodules than on the conventional farms. High rates of N fertiliser addition may have the same effect (see Fig. 11.4.e).

(vi) Were the Conventional and Biodynamic Soils Responding to Nutrient Additions in the Same Manner?

The interactions 'P x management strategy' and 'N x management strategy' were included in the models in Tables 11.2-11.5 to assess whether plants grown in the conventional and biodynamic soils were responding to nutrient additions in the same manner. The trial was designed to investigate this question in a more rigorous manner than was possible for the field data using stepwise regressions (§5.3.e.iii and §10.4.e).

There were only three instances where there was a significant interaction between nutrient addition and management strategy. The effect of P on VAM colonisation was greater on the biodynamic farms, however, as discussed earlier, this was unlikely to be due to differing biological processes being present on conventional and biodynamic farms (§11.1.d.iii). Similarly, the greater effect of N on the rye grass shoot growth was probably due to P limiting the response to N on the biodynamic farms (§11.1.d.iv). Only the increase in nodule numbers in the conventional soil at the second rate of N addition remains inexplicable. Thus, the biological processes in the biodynamic and conventional soils did not appear to be substantially different (see discussion in §12.3.c.iv, v).

11.2. Contribution of VAM Fungi to Growth of Three Pasture Species; Interactions with Phosphorus, Regular Defoliation and Interspecific Competition

11.2.a. Aims

The aims of the glasshouse trial were to investigate the following questions:

• What effects do addition of P, regular defoliation and interspecific competition have on the level of VAM colonisation?

• What contribution do the indigenous VAM fungi make to the growth of the three main pasture species found on the dairy farms; white clover (*Trifolium repens* L.), perennial rye grass (*Lolium perenne* L.) and paspalum (*Paspalum dilatatum* Poir.)?

• Does the addition of P change the contribution of VAM fungi to plant growth?

• Do two factors which generally differ between glasshouse trials and the situation in the field, regular defoliation of shoots and interspecific competition, affect the contribution of VAM fungi to plant growth?

11.2.b. Methods

Soil was collected in November 1996 from the top 50-100 mm of soil in an irrigated permanent pasture paddock on a biodynamic dairy farm in NE Victoria, passed through a 10 mm sieve and large roots or pieces of organic matter removed. The soil was irradiated (§3.7) and Olsen extractable P, total N and pH assessed on a subsample of irradiated and non-irradiated soil by Wesfarmers CSBP (Perth, Western Australia). The trial was a fully crossed factorial experiment with five treatments.

(1) *Plant Species.* The three species were clover, rye grass and paspalum.

(2) *VAM.* The two VAM treatments were with or without addition of VAM inoculum; '+VAM' and '-VAM'. Inoculation consisted of 300 g of irradiated soil being replaced with 300 g of non-irradiated field soil.

(3) *Phosphorus.* The two P treatments were with or without addition of P as 60 mg of $NaH_2PO_4.H_2O$ pot^{-1}; '+P' and '-P'. The P was added as a solution and mixed through the dry soil before it was placed in pots.

(4) *Defoliation.* The two defoliation treatments were with or without regular defoliation; '+defoliation and '-defoliation'. Defoliation occurred every two weeks. For the first occurrence, the grasses were cut with scissors to 20 mm height and the first trifoliate leaf on each clover plant removed. At later defoliations, all plants were cut to 30 mm (Plate 11.2).

5) *Competition*. The two competition treatments were six plants of the same species or two plants each of clover, rye grass and paspalum in each pot; 'monoculture' or 'polyculture'.

All treatments were replicated four times. Squat pots, 175 mm diameter, were filled with 1.2 kg of sieved irradiated soil and sterilised gravel was placed in the bottom to improve drainage. To avoid toxic effects from the irradiation, pots were heavily watered and left for one week before planting and 80 ml of filtrate was added to all pots at planting (§3.7). White clover cv. Kopo, perennial rye grass cv. Yatsynl and paspalum were planted in a 1:1 vermiculite:perlite mix and seedlings transplanted into pots in early December 1996. Pots containing clover plants were inoculated with a mixture of four species of *Rhizobia* suitable for white clover. Basal nutrients, minus P, were applied at planting and again after one month (§3.7).

Shoot material removed during the defoliation treatments was sorted into individual species, dried and weighed (§3.3). The trial was harvested after seven weeks and — after sorting into individual species —shoot dry weight, root wet weight and VAM colonisation were measured for each species present in each pot (§3.3, §3.4 and §3.5). Root wet weights were converted to dry weights (§3.4.b). All measures are expressed on an individual plant basis. Results from each species were examined by ANOVAs using the statistical package JMP®. Parameters fitted were block (1-4), VAM (-VAM, +VAM), P (-P, +P) defoliation (-defoliation, +defoliation) and competition (monoculture, polyculture). Two outliers were removed in the clover root-shoot ratio data and one in each of the rye grass and paspalum root-shoot ratio data.

11.2.c. Results

Prior to irradiation, the soil contained 44 $\mu g\ g^{-1}$ of Olsen extractable P, 8700 $\mu g\ g^{-1}$ of total N and had a pH of 4.8. The irradiated soil contained 43 $\mu g\ g^{-1}$ of Olsen extractable P, 5400 $\mu g\ g^{-1}$ of total N and had a pH of 6.3.

VAM colonisation results are presented in Table 11.8 and Figure 11.9. Addition of VAM inoculum resulted in a relatively low level of colonisation, generally <20%, for all species. Clover was the most highly colonised, with colonisation being significantly reduced by P addition and defoliation. The rye grass was consistently poorly colonised and while P addition had no effect on colonisation, defoliation slightly increased colonisation. The paspalum was more highly colonised than the rye grass, with only P significantly reducing colonisation. Growing plants in a polyculture had no effects on VAM colonisation.

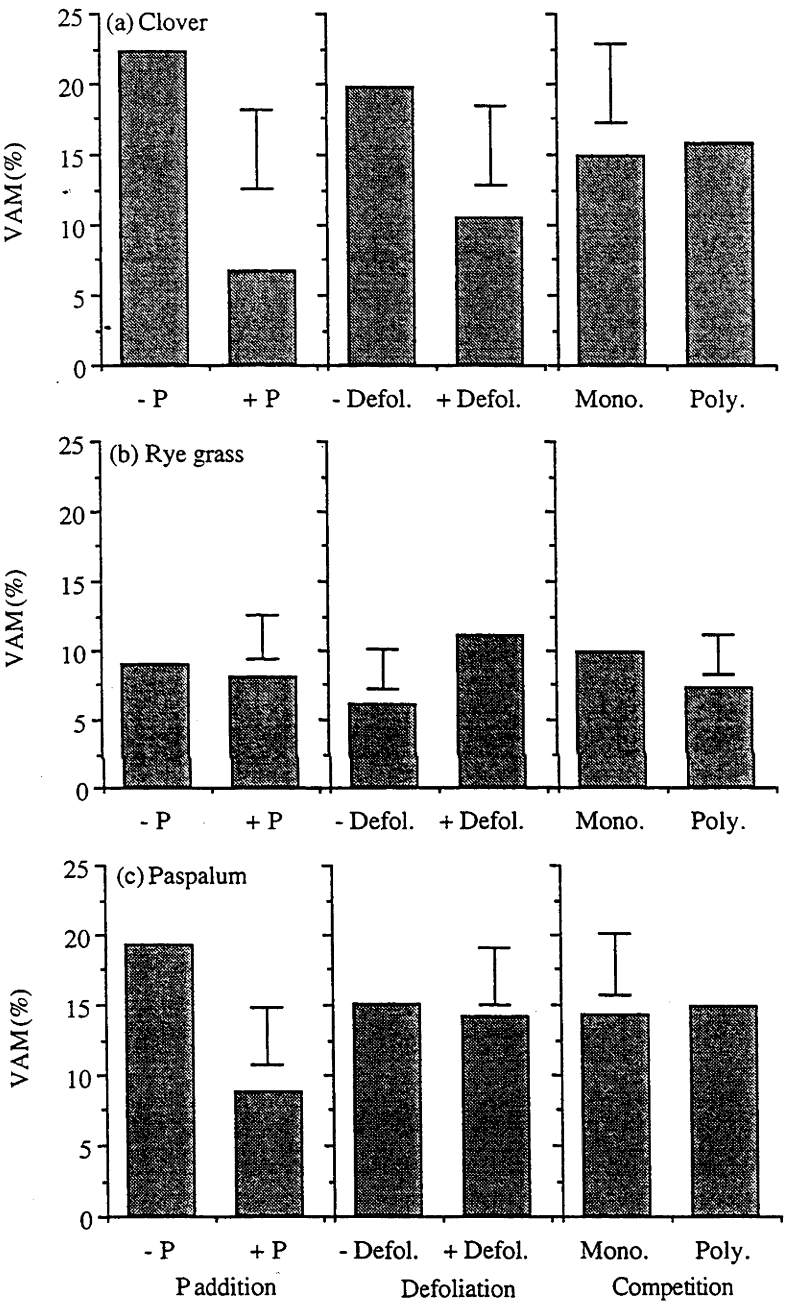


Figure 11.9. Estimated means and LSD at p=0.05 for the percentage of root length colonised by VAM fungi for a) clover, b) rye grass and c) paspalum. Parameters included were P addition (-P, +P), defoliation (-defol., +defol.) and interspecific competition (monoculture (Mono.), polyculture (Poly.)). There were no significant interaction terms.

Table 11.8. Results from ANOVAs of the percentage of root length colonised by VAM fungi in clover, rye grass and paspalum. Parameters included are block (1-4), P addition (-P, +P), defoliation (+defoliation, -defoliation) and competition (monoculture, polyculture). Only treatments where VAM inoculum was added were included. There were no significant interaction terms.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Clover VAM (%)	model	5.3	0.002	0.48	29
	block	2.5	0.09		
	P addition	19.7	0.0002		
	defoliation	7.3	0.01		
	competition	0.3	0.6		
Rye grass VAM (%)	model	2.6	0.06	0.27	26
	block	2.3	0.1		
	P addition	0.2	0.7		
	defoliation	6.0	0.02		
	competition	1.2	0.3		
Paspalum VAM (%)	model	2.8	0.03	0.28	29
	block	0.5	0.7		
	P addition	14.8	0.0009		
	defoliation	0.2	0.7		
	competition	0.02	0.8		

The total dry weight of shoot material produced by each of the three species in the defoliated pots over the course of the trial was 50-75% less than that produced in the non-defoliated pots. Total dry weight was also examined using the models presented in Tables 11.9-11.11, with results being similar to those of shoot dry weight at harvest.

Clover results are presented in Table 11.9 and Figure 11.10. Shoot dry weight was significantly increased by 18% by VAM fungi. Defoliation markedly reduced shoot dry weight. Shoot dry weight was higher in the monoculture than the polyculture, but this difference was reduced by defoliation (Fig. 11.10.a). Defoliation also influenced the response to P, with P addition increasing shoot dry weight without defoliation, but decreasing shoot dry weight when defoliation occurred.

Clover root dry weight was significantly increased by 35% by VAM fungi. Addition of P tended to decrease root weight. Defoliation greatly reduced root dry weight. There was no significant difference in root weight between plants growing in a monoculture or in a polyculture. The root-shoot ratio in clover was not significantly affected by VAM fungi or P addition. Defoliation increased the root-shoot ratio. The root-shoot ratio was higher in the monoculture when defoliation occurred, but did not differ significantly between the monoculture and polyculture when defoliation did not occur.

Table 11.9. ANOVAs of measures of clover growth: shoot dry weight at harvest (g plant^{-1}), root dry weight at harvest (g plant^{-1}), the root-shoot ratio at harvest and shoot dry weight of defoliated plants at week 2 (g plant^{-1}). Parameters included are block (1-4), VAM (-VAM, +VAM), P addition (-P, +P), defoliation (+defoliation, -defoliation) and competition (monoculture, polyculture). All significant interaction terms are included.

Dependent variable	Predictor variable	F-ratio	prob.	r^2	n
Log shoot dry weight	full model	72.2	<0.0001	0.91	61
	block	6.8	0.0006		
	VAM	8.9	0.004		
	P	0.001	1.0		
	defoliation	528.4	<0.0001		
	competition	52.5	<0.0001		
	defoliation x P	8.8	0.005		
	defoliation x competition	20.5	<0.0001		
Log root dry weight	full model	59.6	<0.0001	0.87	0.61
	block	5.7	0.002		
	VAM	14.6	0.0004		
	P	3.3	0.07		
	defoliation	378.0	<0.0001		
	competition	1.9	0.8		
Root-shoot ratio	full model	5.9	<0.0001	0.40	59
	block	0.3	0.8		
	VAM	2.9	0.09		
	P	2.1	0.1		
	defoliation	6.4	0.01		
	competition	22.4	<0.0001		
	defoliation x competition	15.9	0.0002		
Dry weight of shoots removed during defoliation at week 2	full model	1.6	0.2	0.1	30
	block	2.1	0.1		
	VAM	0.7	0.4		
	P	2.8	0.1		
	competition	0.04	0.8		

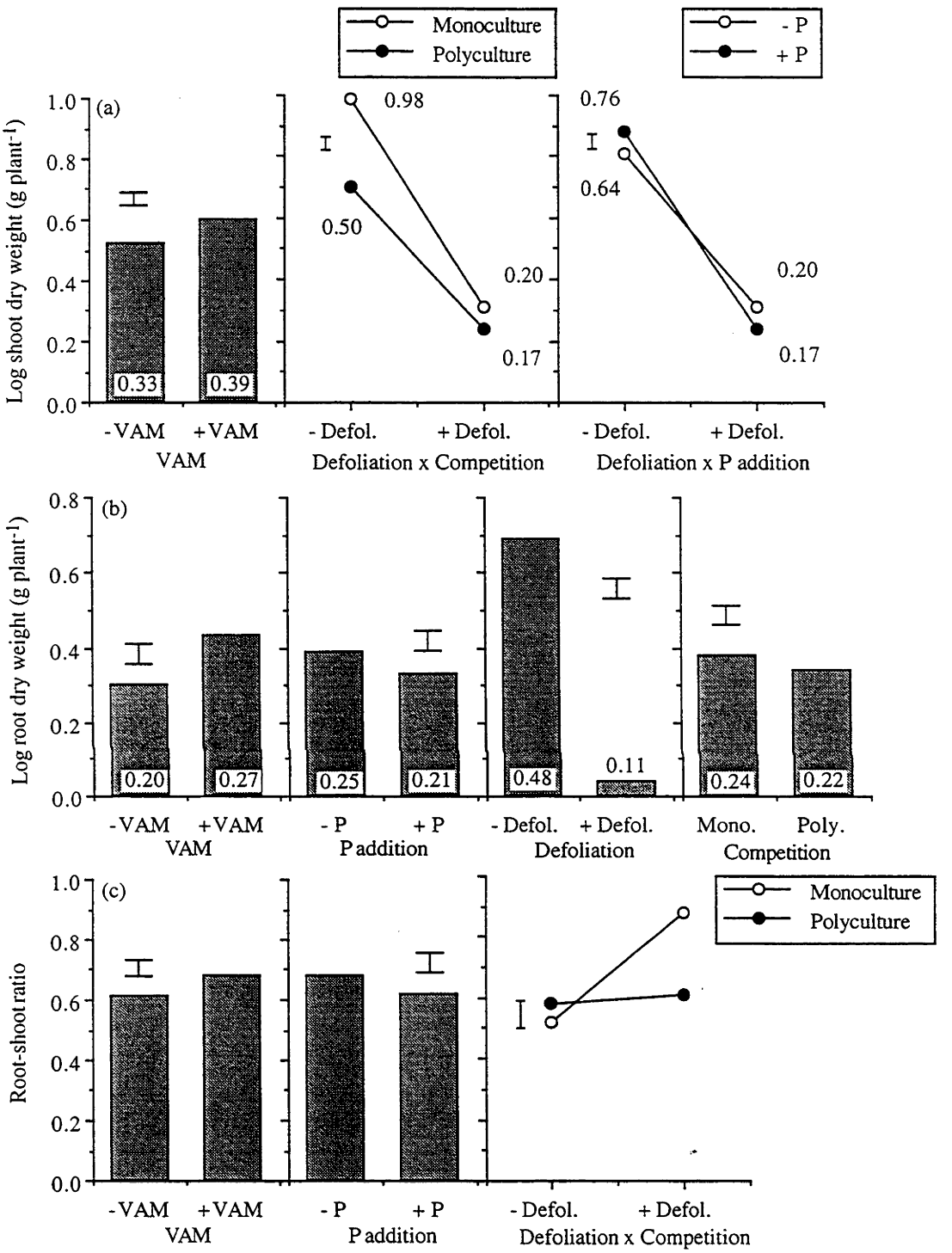


Figure 11.10. Estimated means and LSD at p=0.05 for clover: a) shoot dry weight at harvest, b) root dry weight at harvest and c) the root-shoot ratio at harvest. Parameters included were inoculation with VAM fungi (+VAM, -VAM), P addition (-P, +P), defoliation (-defol., +defol.) and interspecific competition (monoculture (Mono.), polyculture (Poly.)). Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column or close to the data point on the interaction graphs. All significant interaction terms are shown.

The dry weight of the shoot material removed during defoliation at week 2 showed no significant treatment effects (Table 11.9). A significant effect from VAM fungi did not appear until week 10, but then increased between week 10 and harvest at week 12 (Fig. 11.11).

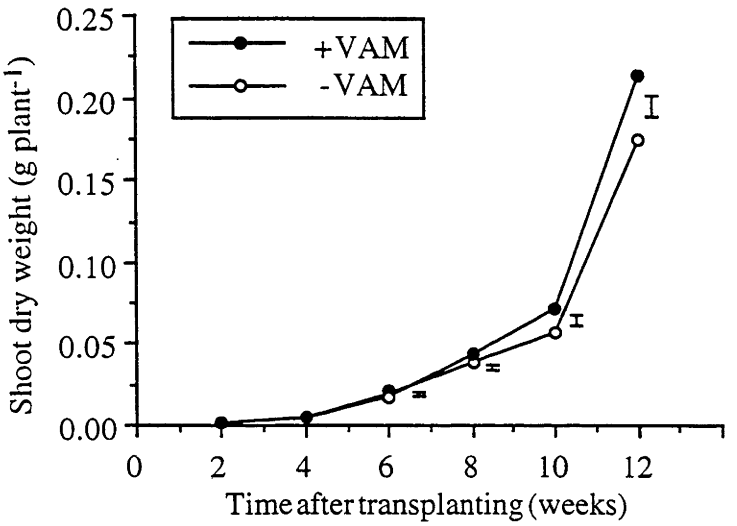


Figure 11.11. Estimated means and LSD at p=0.05 from the VAM treatment (+ VAM, -VAM) for the dry weight of shoots removed during the defoliation treatment from clover plants at weeks 2, 4, 6, 8 and 10 and the shoot dry weight at week 12 (harvest).

Table 11.10. ANOVAs of measures of rye grass growth: shoot dry weight at harvest (g plant^{-1}), root dry weight at harvest (g plant^{-1}) and the root-shoot ratio at harvest. Parameters included are block (1-4), VAM (-VAM, +VAM), P addition (-P, +P), defoliation (+defoliation, -defoliation) and competition (monoculture, polyculture). All significant interaction terms are included.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Log shoot dry weight	full model	158.2	<0.0001	0.95	60
	block	3.7	0.02		
	VAM	0.2	0.6		
	P	6.4	0.01		
	defoliation	1096.7	<0.0001		
	competition	1.1	0.3		
Log root dry weight	full model	69.9	<0.0001	0.91	57
	block	1.9	0.1		
	VAM	9.3	0.004		
	P	0.03	0.86		
	defoliation	525.8	<0.0001		
	competition	0.4	0.5		
	defoliation x competition	8.6	0.005		
Root-shoot ratio	full model	7.9	<0.0001	0.50	56
	block	0.6	0.6		
	VAM	20.4	<0.0001		
	P	13.2	0.0007		
	defoliation	0.4	0.5		
	competition	3.9	0.05		
	defoliation x competition	19.3	0.0001		

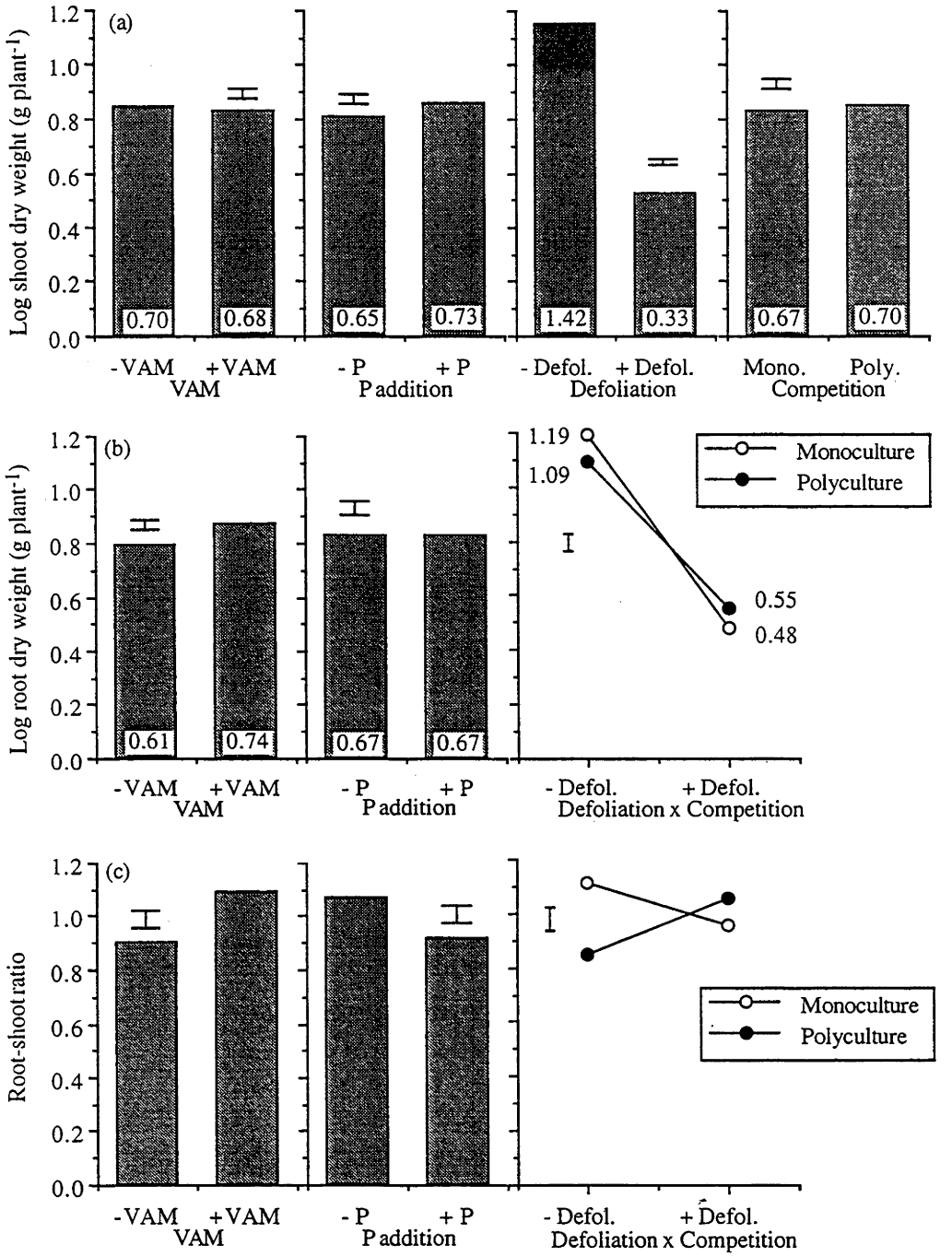


Figure 11.12. Estimated means and LSD at p=0.05 for rye grass: a) shoot dry weight at harvest, b) root dry weight at harvest and c) the root-shoot ratio at harvest. Parameters included were inoculation with VAM fungi (+VAM, -VAM), P addition (-P, +P), defoliation (-defol., +defol.) and interspecific competition (monoculture (Mono.), polyculture (Poly.)). Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column or close to the data point on the interaction graphs. All significant interaction terms are shown.

Rye grass shoot dry weight was greatly decreased by defoliation, slightly increased by P addition and was not influenced by VAM fungi or competition (Table 11.10 and Fig. 11.12). Root dry weight was significantly increased by 18% by VAM fungi and greatly decreased by defoliation. There was a significant interaction between defoliation and competition, with root dry weight being greater in the monoculture when no defoliation occurred, but greater in the polyculture when defoliation occurred. The root-shoot ratio was increased by VAM colonisation, decreased by P addition and was greatest in the monoculture when defoliation occurred, but greater in the polyculture when there was no defoliation

Results for paspalum are contained in Table 11.11 and Figure 11.13. Both shoot and root growth tended to be slightly increased by VAM, were not influenced by P, were markedly reduced by grazing and were slightly higher in the polyculture. The root-shoot ratio was reduced by defoliation and competition, with the defoliation effect being more marked in the monoculture.

Table 11.11. ANOVAs of measures of paspalum growth: shoot dry weight at harvest (g plant^{-1}), root dry weight at harvest (g plant^{-1}) and the root-shoot ratio at harvest. Parameters included are block (1-4), VAM (-VAM, +VAM), P addition (-P, +P), defoliation (+defoliation, -defoliation) and competition (monoculture, polyculture). All significant interaction terms are included.

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
Log shoot dry weight	full model	154.2	<0.0001	0.95	60
	block	2.7	0.06		
	VAM	3.5	0.07		
	P	1.3	0.3		
	defoliation	1017.1	<0.0001		
	competition	30.4	<0.0001		
Log root dry weight	full model	211.5	<0.0001	0.96	60
	block	5.0	0.004		
	VAM	8.7	0.005		
	P	0.003	1.0		
	defoliation	1439.7	<0.0001		
	competition	10.0	0.004		
Root-shoot ratio	full model	10.2	<0.0001	0.56	59
	block	1.1	0.4		
	VAM	1.4	0.2		
	P	0.5	0.5		
	defoliation	48.0	<0.0001		
	competition	11.0	0.0002		
	defoliation x competition	15.0	0.0003		

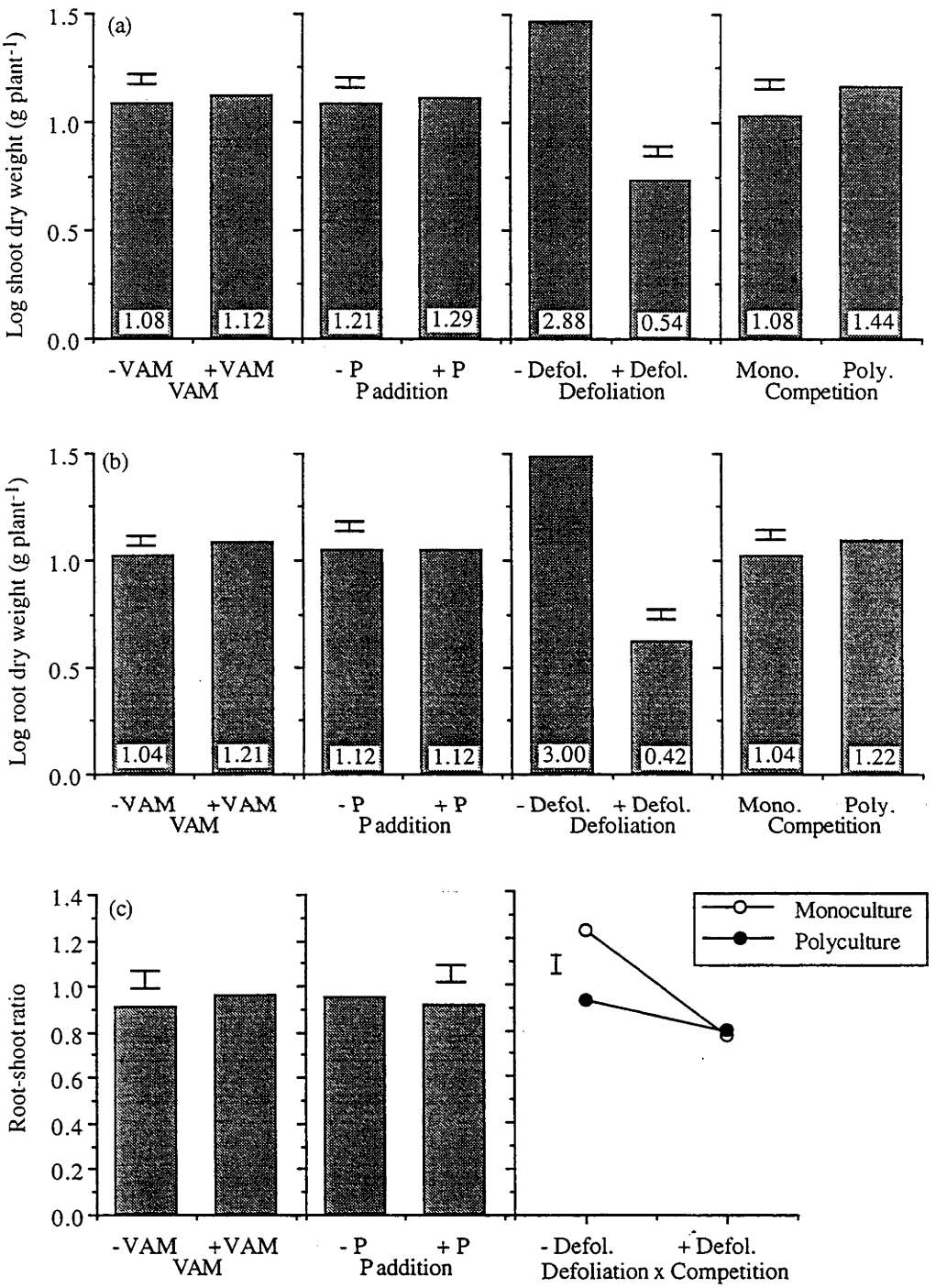


Figure 11.13. Estimated means and LSD at p=0.05 for paspalum: a) shoot dry weight at harvest, b) root dry weight at harvest and c) the root-shoot ratio at harvest. Parameters included were inoculation with VAM fungi (+VAM, -VAM), P addition (-P, +P), defoliation (-defol., +defol.) and interspecific competition (monoculture (Mono.), polyculture (Poly.)). Means from data which were log transformed were first multiplied by ten to eliminate negative values and untransformed means in grams are given at the base of each column. All significant interaction terms are shown.

11.2.d. Discussion

(i) Soil Nutrient Concentrations

Irradiation substantially reduced total N in the soil, however, this would have had little effect on results, as N was applied in the basal nutrients at a rate to ensure it was not limiting plant growth. Soil from a biodynamic farm (Pair B) was used to ensure a low extractable P concentration, however, extractable P was 43 $\mu g\ g^{-1}$; higher than anticipated. This high concentration may have resulted from the method of soil sampling. As the paddock was very dry, it was difficult to dig deeper than 50 mm, thus soil was primarily taken being from the top of the profile where P concentrations are higher. Moreover, the soil mat and other organic matter was also included and this would have begun to decay and release P by the time the soil had been sieved and irradiated.

Small *et al.* (1994a) sampled the top 50 mm of soil on the NE Victorian dairy farms in November 1991. Average soil extractable P, excluding the soil mat, was 20 $\mu g\ g^{-1}$ on the biodynamic farms and 38 $\mu g\ g^{-1}$ on the conventional farms. Thus the concentration of extractable P in the glasshouse trial was similar to that on the conventional farms, but higher than would be expected on the biodynamic farms.

(ii) VAM Colonisation Levels

Both the clover and paspalum had approximately 15-20% of root length colonised by VAM fungi, while rye grass had half this level. Addition of P decreased VAM colonisation in clover and paspalum, but had no effect on the rye grass; again indicating a weaker relationship between P and VAM colonisation in rye grass (see Fig. 11.8).

Defoliation decreased VAM colonisation in clover, increased it in rye grass and had no effect in paspalum. Daft and El-Giahmi (1978) reported that periodic defoliation reduced the level of VAM colonisation by 50% in maize, tomatoes, grasses and alfalfa; no root weights were reported. They suggest that the decrease in VAM colonisation is due to a reduction in availability of host plant photosynthate (Daft and El-Giahmi 1978). Interpretation of defoliation experiments is complex as VAM (%) is a product of the growth rate of both the VAM fungi and roots. The rye grass and paspalum had root a biomass 3-5 times greater than the clover, therefore, when plants were not defoliated, VAM colonisation may have been unable to keep pace with root growth. Consequently, defoliation may have slowed root growth more than fungal growth, resulting in no effect — or a positive effect — on VAM (%). It is also possible that the effects of defoliation on VAM colonisation resulted from an interaction with the effects of defoliation on plant P.

Interspecific competition (polyculture) between host plants had no effect on VAM (%) in comparison to intraspecific competition (monoculture). Growth of clover was negatively affected by being in a polyculture when defoliation did not occur, however, presumably the competition did not reduce the photosynthetic rate sufficiently to influence the VAM colonisation level.

(iii) Influence of VAM Fungi on Plant Growth

VAM inoculation increased shoot growth by 18% for clover, -3% for rye grass and 4% for paspalum, while root growth was increased by 35% in clover, 21% in rye grass and 16% in paspalum. Thus it would appear that the dependency of clover on VAM fungi > paspalum > rye grass. The root-shoot ratio was increased by VAM colonisation in all three species, which was unexpected as VAM colonisation is generally reported to reduce the root-shoot ratio (Allsopp and Stock 1992; Koide 1991; Smith and Gianinazzi-Pearson 1990). Section 12.1.b.i contains further discussion of dependency and the effects of VAM colonisation on the root-shoot ratio.

The relatively small growth increase in the clover presumably resulted from the high concentration of soil extractable P. The growth response of clover to VAM fungi will decrease to zero, or become negative, as P becomes more available (Hall 1978). Rapid plant growth and lower inoculum levels in the glasshouse trial resulted in colonisation levels being lower than in the field (Fig. 10.1); this may also have reduced the growth response to VAM colonisation.

There was no significant interaction between VAM and P addition, although VAM colonisation did tend to have less effect on clover growth when P was supplied (results not shown). This interaction may have been significant if soil extractable P had been lower. There was also no significant interaction between VAM and defoliation. Daft and El-Giahmi (1978) found periodic defoliation of maize and tomatoes reduced the increase in shoot growth in response to VAM colonisation from 80% to 27-50%. This corresponded with a decrease in VAM colonisation, but could also have been the result of the plant growth rate being increased and other factors, besides P, limiting growth (the total biomass produced was higher in the defoliated treatments). In the current trial, less total biomass was produced in the defoliated treatments, resulting in less demand for nutrients, although this may have been offset by the smaller root biomass of the defoliated plants.

There was also no interaction in the trial between VAM and competition. VAM fungi have been found to influence the outcome of interspecific competition in a number of cases (Crush 1995; Fitter 1977; Grime *et al.* 1987). Hall (1978) used a low-P soil and found that, after 90 days, VAM fungi produced a three-fold increase in clover weight when clover was grown alone and a 24-fold increase when grown with rye grass. This was due to rye grass suppressing clover growth more strongly in the non-

VAM treatments. However, at the higher levels of P addition, VAM fungi either had no effect on clover growth or reduced growth (Hall 1978). Thus, although there was no interaction between VAM and competition in the current trial, it is likely that this would occur for the clover if soil extractable P was lower.

Results from the defoliated clover plants presented in Figure 11.11 indicated that VAM colonisation was not resulting in a net increase in plant growth until week 8. This, again, must reflect the relatively high soil extractable P; presumably P did not become limiting immediately to the plants.

(iv) Other Factors Influencing Shoot Growth

In all three species, addition of P had a slight positive effect on shoot growth and no effect, or a negative effect, on root growth. This is consistent with the results from the glasshouse trial presented in section 11.1, where P was not limiting plant growth in the majority of soils (Figs. 11.4 and 11.6). Defoliation greatly reduced the final biomass of roots and shoots for all three species and it also greatly reduced the growth rate of the plants, resulting in less total biomass. Interspecific competition (polyculture) reduced clover growth, had no effect on the rye grass and greatly increased paspalum growth. The negative effect on the clover was reduced when defoliation occurred (Fig. 11.10.a), indicating that to maintain clover as part of a pasture sward in the field, regular grazing may need to occur.

11.3. Contribution of VAM Fungi to Growth of White Clover; Effects of VAM Species and Plant Density

Two additional small glasshouse trials, using only white clover, were conducted to examine other factors which may influence the relevance of glasshouse trials to field conditions; VAM species and plant density. The trials were intended only as a preliminary examination of these factors and were conducted with smaller pots and fewer plants in each pot than in the trial presented in section 11.2.

11.3.a. Aims

The aims of the two glasshouse trials were to investigate the following questions.

- Will the effect of VAM fungi on clover growth vary with the species of VAM fungi present?

- Will the effect of VAM fungi on clover growth vary with the density of the clover plants?

11.3.b. Methods

These trials were run simultaneously to the trial reported in section 11.2 and unless stated otherwise, methods were identical. Square pots, 70 mm diameter, were filled with 200 g of soil. Two trials were conducted.

Trial A: The Effect of VAM Species on Clover Growth. Two plants were planted in each pot and four treatments applied: no addition of VAM inoculum 'control'; VAM fungi indigenous to the soil 'field'; *Glomus intraradices*; and *Scutellospora calospora.* The indigenous VAM fungi were introduced through replacing 50 g of irradiated soil with 50 g of non-irradiated soil. *Glomus* and *Scutellospora* were introduced by mixing 5 g of inoculum — sand, roots and spores — into each planting hole. Inoculum was provided by Dr Sally Smith, Waite Institute, Adelaide.

Trial B: The Interaction between VAM Fungi and Clover Density. Clover was grown at four densities — one, two, four or eight plants in each pot — with and without inoculation with indigenous VAM fungi. The fungi were introduced as in Trial A.

Treatments were replicated four times, with each set of replicates forming a randomised block in the glasshouse. White clover was used in all trials and *Rhizobium* bacteria were added as described in section 11.2.b. Half strength basal nutrients, minus P, were applied at transplanting and three weeks after sowing (§3.7). Filtrate was applied at transplanting (§3.7). Both trials were harvested after 10 weeks and shoot dry weights, root wet weights and VAM colonisation measured (§3.3, §3.4 and §3.5). Root

wet weight measures were converted to dry weights (§3.4.b). All trials were analysed using the statistical package JMP®.

11.3.c. Results

The results from Trial A are presented in Table 11.13 and Figure 11.14. *Glomus, Scutellospora* and the indigenous VAM species all caused colonisation. The level of colonisation in the *Glomus* treatment was lower than in the other two treatments (Fig. 11.14.a). Shoot dry weight was significantly increased by around 30% by all three VAM treatments. Root dry weights were very variable and were not significantly influenced by the addition of VAM fungi.

Table 11.13. Results from ANOVAs of Trial A: percentage of root length colonised by VAM fungi, shoot dry weight (g plant^{-1}) and root dry weight (g plant^{-1}). The parameter varied was VAM (control, field, *Glomus, Scutellospora*). The control was excluded from the analysis VAM (%).

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM(%)	full model/VAM	8.3	0.009	0.57	12
Shoot dry weight	full model/VAM	6.0	0.01	0.51	15
Root dry weight	full model/VAM	0.9	0.5	-0.01	15

Table 11.14. Results from ANOVAs of Trial B: the percentage of root length colonised by VAM fungi, shoot dry weight (g plant^{-1}) and root dry weight (g plant^{-1}). Parameters included were block (1-4), VAM (control, field, *Glomus, Scutellospora*) and density (1, 2, 4, 8 plants pot^{-1}). The control was excluded from the analysis of VAM (%).

Dependent variable	Predictor variable	F-ratio	Prob.	r^2	n
VAM(%)	full model	2.1	0.15	0.30	16
	block	1.9	0.21		
	density	2.3	0.14		
Log shoot dry weight	full model	59.8	<0.0001	0.93	32
	block	1.0	0.4		
	density	137.6	<0.0001		
	VAM	3.1	0.09		
Log root dry weight	full model	12.5	<0.0001	0.72	32
	block	1.4	0.3		
	density	26.8	<0.0001		
	VAM	3.0	0.09		

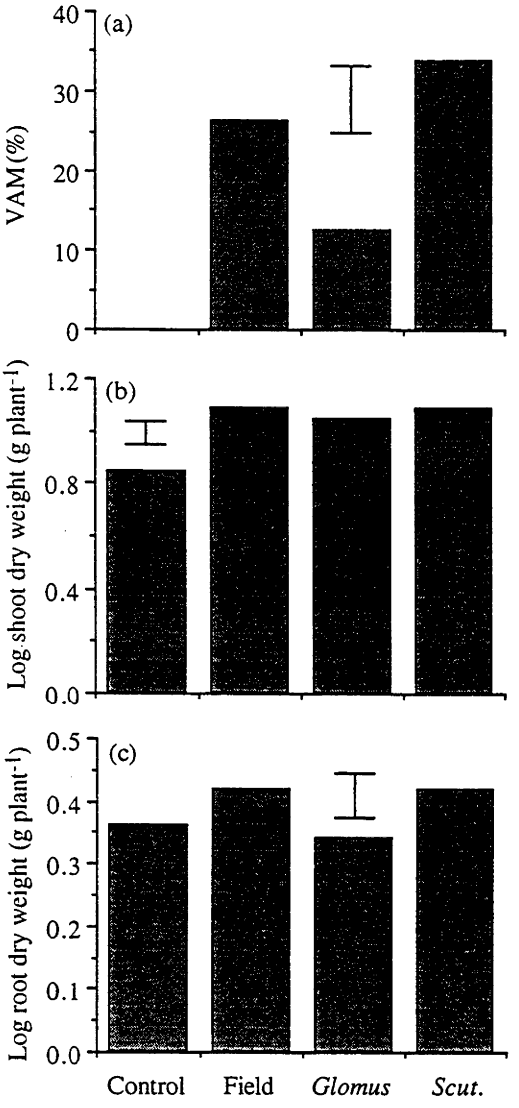


Figure 11.14. Effects of VAM species (control, field, *Glomus*, *Scutellospora*) on a) the percentage of root length colonised by VAM fungi, b) log shoot dry weight and c) log root dry weight; estimated means and LSD at p=0.05.

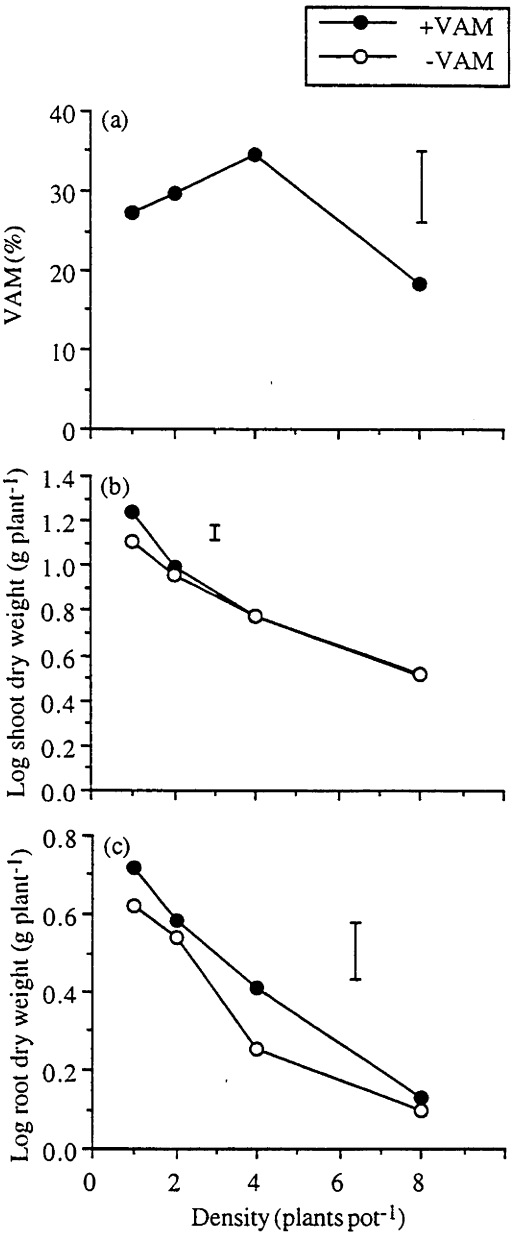


Figure 11.15. Effects of plant density (1, 2, 4, 8) on a) the percentage of root length colonised by VAM fungi; and the effects of plant density (1, 2, 4, 8) and VAM colonisation (+VAM, -VAM) on b) log shoot dry weight and c) log root dry weight; estimated means and LSD at p=0.05.

The results from Trial B are presented in Table 11.14 and Figure 11.15. VAM colonisation was not significantly affected by density, although colonisation was lower at the highest density. Individual plant shoot and root dry weights decreased as density increased, indicating that the plants at the higher densities were under competitive stress. There was no significant interaction between VAM and density, however at the lowest density VAM colonised plants were significantly heavier and this effect disappeared as density increased. VAM colonisation did not significantly effect root weight.

11.3.d. Discussion

All three inoculum types had an identical effect on plant growth, indicating that the indigenous VAM species were no better or worse at increasing plant growth than the non-indigenous *Glomus* and *Scutellospora*. This result is consistent with the outcomes of the wheat glasshouse trial (§7.3). The lower level of colonisation by the *Glomus* may result from its preference for a less acidic soil (S. Dickson, pers. comm.). Thus, its ability to enhance shoot growth to a similar level to the other VAM species must reflect a difference in other characteristics, such as the form of external hyphae (Abbott and Robson 1985b; Abbott *et al.* 1992).

The results from Trial B indicated that VAM colonisation levels may decrease at high root densities. This was not found in the wheat glasshouse trial (§7.3), however, the smaller pots in the present trial resulted in a much higher root density than in the wheat trial. Bååth (1984) also found VAM colonisation to decrease at high root densities, perhaps an indication of individual plants having a lower photosynthetic rate due to shading from neighbours. Colonisation by VAM fungi increased shoot biomass by 33% at the lowest density, 8% at the next density and had no significant effect at the two highest densities. A similar result was found by Facelli (unpublished data, in Smith and Read 1997). Presumably, at the higher plant densities, there was no advantage from VAM hyphae exploring extra soil volume, as the root density was high enough to extract all available nutrients. VAM fungi and root density is discussed further in section 12.1.b.ii. The results in Fig. 11.15.b might also indicate that the VAM hyphae are accessing the same forms of nutrients in the soil as the plant roots. If the hyphae were accessing nutrients unavailable to plants, the growth enhancing effect of the fungi should persist at the higher densities.

11.4. Conclusions from the Glasshouse Trials using Dairy Farm Soils

- The VAM inoculum potential was higher in soil from biodynamic farms.

- The VAM inoculum potential differed substantially between host plant species, but for clover was strongly correlated with field colonisation levels.

- The plant growth potential in unfertilised soil was similar in soil from three conventional and two biodynamic farms, but it was lower in soil from one biodynamic farm due to low soil extractable P.

- The differences in plant growth potential were greatest in the VAM dependent host plants.

- P addition decreased VAM colonisation in clover but not rye grass.

- N addition had no effect in VAM colonisation.

- Defoliation reduced VAM colonisation in clover, but not rye grass or paspalum.

- P addition generally did not increase plant growth.

- N addition increased growth of clover and rye grass.

- VAM colonisation levels were strongly negatively correlated to shoot P.

- The relationship between VAM (%) and shoot P was identical for plants grown in conventional and biodynamic soil.

- Plants growing in conventional and biodynamic soils responded in the same manner to nutrient additions.

- Levels of *Rhizobium* nodulation tended to be increased by P and decreased by N addition.

- VAM colonisation increased shoot growth in clover by 18% and had little effect on rye grass or paspalum shoot growth in a biodynamic soil with relatively high levels of extractable P.

- VAM colonisation increased root growth in clover, rye grass and paspalum.

- Addition of P, regular defoliation and interspecific competition did not change the effect of VAM fungi on plant growth.

- Indigenous VAM fungi and two pure cultures had an identical effect on growth of clover.

- The positive effect of VAM fungi on clover shoot growth decreased to zero with increasing plant density.

- The effects of VAM colonisation on shoot growth may be greater in the field on biodynamic farms than in the glasshouse trial in section 11.2, due to soil extractable P being be significantly lower in the field.

Part E

General Discussion

Chapter Twelve

General Discussion

This chapter synthesises the major findings of the project by addressing each of the topics originally listed at the conclusion of Chapter 1 in Figure 1.3. It is therefore divided into three sections: the form of the VAM-host plant relationship, the effects of the VAM symbiosis on ecosystem functioning and comparisons of the functioning of conventional and alternative agricultural systems. The chapter concludes with the overall project conclusions. Detailed discussion of specific results was contained in earlier chapters and is generally not reiterated. Suggestions for further research are made throughout the chapter and summarised at the end.

12.1. The VAM Fungi-Host Plant Relationship

Section 12.1 examines the ecology of VAM fungi at the VAM fungi-host plant relationship scale, using results from the mixed and dairy systems. Section 12.1.a considers the factors which influence the level of VAM colonisation, while the effects of VAM colonisation on plant growth — and the relevance to field conditions of results obtained from glasshouse trials — are discussed in section 12.1.b. Section 12.1.c discusses the form of the host plant-VAM fungi relationship under field conditions and speculates on how this relationship may have evolved.

12.1.a. Which Environmental and Farm Management Factors Control the Level of VAM Colonisation?

This section begins by considering various methods for measuring the level of VAM colonisation in field samples. The relationships between VAM colonisation levels and both environmental factors and farm management practices are then discussed, commencing with the main variables included in this project; soil nutrients (P and N) and water. The section concludes with a discussion of the spatial and temporal scales at which these relationships were evident.

(i) Methods for Assessing the Level of VAM Colonisation in the Field

VAM colonisation levels are generally measured as the percentage of root length colonised, VAM (%); see Giovanetti and Mosse (1980). Difficulties with correlating VAM colonisation and plant growth in the field may result from VAM (%) not providing an accurate assessment of mycorrhizal activity (§1.5) and, therefore VAM intensity was also measured in this project. In the wheat crops, VAM intensity magnified some trends shown by VAM (%), however, in both crop and pasture samples, there was a strong positive correlation between VAM intensity and VAM (%). The proportion of colonisation consisting of arbuscules is another alternative measure of VAM colonisation (Smith and Gianinazzi-Pearson 1990), but was not trialed in this project. However, all measures of VAM fungi, based on non-vital staining, do not indicate whether the fungi are active. Even if vital staining (Hamel *et al.* 1990; Soderström 1977) is used on field samples, there is still no measure of the contribution of the fungi to plant growth.

Root length is often measured in studies of agricultural crops — for example Pearson *et al.* (1991) and Rickert *et al.* (1987) — but is rarely measured in field studies of VAM fungi. Yet differences in VAM (%) may not be reflected in the length of colonised roots present if more highly colonised plants possess fewer roots. In the wheat crops, root length and VAM (%) were greater on the alternative farms and thus measuring the length of root colonised by VAM fungi magnified the differences found when VAM (%) was measured.

Overall, the additional effort required for the two alternative methods used in this project for assessing VAM colonisation levels on field samples did not result in a commensurate increase in information. Moreover, the results indicated that VAM (%) is adequate for identifying broad-scale relationships in the field between VAM colonisation and environmental factors or farm management practices.

(ii) Soil and Plant Nutrient Concentrations

Phosphorus

Soil extractable P was consistently strongly negatively correlated with VAM colonisation. Consequently, farms which regularly applied soluble P fertilisers had consistently lower VAM colonisation. This correlation was supported experimentally in glasshouse trials using both mixed and dairy farm soils (Figs. 7.3 and 11.4 and see also Fig. 5.17).

It is well accepted that P addition generally results in reduced VAM colonisation (Smith and Read 1997). This may be due to direct effects on the fungi, as P may increase the time taken for inoculum to colonise roots (Amijee *et al.* 1993a) and may inhibit the branching of hyphae produced by spores (Douds *et al.* 1996). However, indirect effects on the fungi — mediated by the host plant — may often be more important. High concentrations of P in the plant slows both the growth of VAM fungi in the roots and the formation of secondary colonisation points (Bruce *et al.* 1994).

Shoot P in clover plants grown in the glasshouse had a strong negative correlation with VAM colonisation (Fig. 11.7, r^2=0.97 using treatment means), strongly indicating that the main mechanism limiting VAM colonisation was the concentration of P in the plant. However, this does not necessarily mean that the effect of P on VAM colonisation was solely mediated through the plant, as this relationship could be correlated with indirect effects on spore germination and growth.

In field samples collected during this project, the correlation between VAM colonisation and both soil extractable P and plant P were examined. For clover from the dairy pastures, soil extractable P and plant P explained approximately the same amount of variation in VAM (%) (Table 10.5). Root P was examined on a subset of dairy farms and had a stronger relationship with VAM colonisation than shoot P (Fig. 10.7). This supports the hypothesis that the concentration of P in the portion of root system being colonised determines the level of VAM colonisation (Lu *et al.* 1994) and is consistent with the effects of P being mediated through the effects of the phospholipid content of root cells on root cell membrane permeability and exudation of carbohydrates (Graham *et al.* 1981). The stronger correlations between shoot P and VAM colonisation in the glasshouse trials than in the field suggest that factors in the field, such as grazing or disease, disrupt the relationship between shoot P and root P.

In the wheat crops at tillering, shoot P explained only half the amount of variation in VAM colonisation as soil extractable P (Table 5.8). This may indicate a poor relationship between shoot and root P, due to the annual nature of the crop. Wheat root growth and P absorption peak before shoot growth; 50-60% of total P is absorbed when shoots have developed only 20-35% of total dry matter (Römer and Schilling 1986).

Nitrogen

In wheat on the mixed farms, soil total N had a small positive effect on VAM colonisation and a large positive effect on VAM intensity (Table 5.8), while in pastures, shoot N again correlated positively with VAM colonisation (Table 6.5; soil total N not measured). For the dairy farms, in the field and in glasshouse trials, there was no relationship between VAM colonisation and N, reflecting the higher total N in the dairy farm soils. Soil total N ranged from 700-1700 $\mu g\ g^{-1}$ on the mixed farms and 3500-5800 $\mu g\ g^{-1}$ on the dairy farms (Tables 5.3 and 10.1). As the wheat was not N-limited, while the dairy pastures were N-limited, it appears that the concentration at which N becomes limiting can differ for VAM fungi and their host plant.

The effects of N addition on VAM colonisation are reported to vary from negative (Buwalda and Goh 1982, Ellis *et al.* 1992) to neutral (Azcón *et al.* 1992; Ryan 1992) and positive (Johnson 1993; Vejsadová *et al.* 1989). Interactions between P and N have also been reported, with P having a greater effect on VAM colonisation when N was deficient (Sylvia and Neal 1990) and addition of N negatively affecting colonisation only when P was abundant (Bååth and Spokes 1989). Overall, the mechanisms behind the response of VAM fungi to N addition have not been sufficiently researched to allow generalisation. This project simply indicated that N has the potential to limit growth of VAM fungi, even when the growth of the host plant is not N-limited.

(iii) Water

The cereal crops sampled during the 1994 drought (Chapter 8) indicated that water stress can markedly restrict VAM colonisation; P accumulation occurred in some crops, but was not present in all crops with low VAM colonisation (§8.2.c.i). The lower VAM colonisation on irrigation check banks on the dairy farms probably also resulted from water stress (§10.4.b.ii and Fig. 10.9). Mohammad *et al.* (1995) also found that water stress — watering pots to field capacity every three days as opposed to every day — resulted in VAM colonisation in wheat being halved. In SW Queensland, Armstrong *et al.* (1992) found less pasture species were colonised by VAM fungi in winter after a long drought, than the following summer after good rainfall.

As with P, water stress may reduce VAM colonisation through either a direct effect on the growth of the VAM fungi or an indirect effect mediated through the host plant. The 1994 sampling was not designed as a drought experiment and no measures of soil matric potential were made. However, soil water between the matric potentials of -0.01 and -1.5 MPa is generally considered to be available to plants, while seasonally dry field soil may reach a matric potential of -35 MPa (Jasper *et al.* 1993). A soil matric potential of -0.19 MPa was found to be optimal for initial VAM colonisation by Reid and Bowen (1979), while spores of VAM fungi indigenous to Western Australia had reduced rates of germination and hyphal extension at matric potentials < -1.5 MPa, with spores ceasing to germinate — although remaining viable — at < -5 MPa (Tommerup 1984). The effectiveness of other forms of inoculum, such as hyphae (Jasper *et al.* 1993) and root fragments, is also likely to be reduced under dry conditions. Thus the dry conditions prior and subsequent to sowing in 1994 probably resulted in reduced germination and growth of VAM spores and other inoculum. When colonisation of crops did occur, growth of the fungi within the root may have been restricted through water stress lowering the rate of photosynthesis of the host plant (see §12.1.a.iv).

(iv) Light

Low light levels may reduce VAM colonisation (Bethlenfalvay and Pacovsky 1983). Low light levels appeared responsible for the negative relationship between VAM colonisation level and wheat growth in the glasshouse trial in section 7.2. However, it is difficult to determine whether VAM colonisation was reduced by the low light levels; while colonisation was lower than in the second wheat trial (§7.3) (25-40% compared with 40-60%), the first trial used field soil as inoculum while the second trial used pure cultures of *Glomus* and *Scutellospora.* Son and Smith (1988) conducted a glasshouse experiment using leeks (*Allium cepa.* L.) and found that a combination at low light — mean daily irradiance of 250 µmol m^{-2} s^{-1} — and high P reduced VAM colonisation; although this didn't occur in a second trial conducted in a growth cabinet. Smith and Gianinazzi-Pearson (1990) also found that low light levels reduced VAM colonisation only when P levels were high. As noon maximum light levels were 200-400 µmol m^{-2} s^{-1} in the first wheat trial in this project, it is likely that the mean daily level would be < 250 µmol m^{-2} s^{-1}; however soil extractable P was relatively low. It seems unlikely that light levels in the field would be consistently low enough to effect VAM colonisation levels in winter wheat; particularly given the low soil extractable P on the mixed farms.

(v) Grazing

Daft and El-Giahmi (1978) reported large decreases in VAM colonisation after defoliation, presumably due to a reduction in the amount of photosynthate available to

the roots and the fungi. In this project, the effects of defoliation on VAM colonisation were measured in a glasshouse trial and were found to vary with host plant. However, interpretation of the results was complex due to the effects of defoliation on root length (§11.2.d.ii).

In field trials, Wallace (1987) and Trent *et al.* (1988) found grazing by ungulates to have no effect on VAM colonisation. Under field conditions, factors other than supply of photosynthate — such as high P or water stress (Chapters 8 and 10) — may often limit VAM colonisation. However, Wallace (1981) found a positive relationship between grazing intensity and VAM colonisation.

(vi) Species of VAM Fungi Present

Colonisation levels and rates differ between species of VAM fungi (Figs. 7.3 and 11.14.a). This will not unduly affect interpretation of results when comparing VAM (%) between sites in the field if — as was the case on the dairy farms — a large number of species are present at each site, or if each site has the same species present. However, comparisons between sites where different species of VAM fungi are present may need to consider the colonisation characteristics of the different species, particularly if a different single species dominates at each site, as reported by Jasper *et al.* (1979).

(vii) Competition between Host Plants

The effect of intraspecific competition on VAM colonisation was assessed through the inclusion of two densities of wheat in a glasshouse trial (§7.3). Density had no effect on VAM colonisation and, as root densities were higher than in the field, it is unlikely that root density influenced VAM (%) in the field crops. However, high plant density did reduce VAM colonisation in white clover (Fig. 11.15). Bååth and Hayman (1984) also reported lower VAM colonisation at higher plant densities; as each seedling was individually inoculated, the effect appeared to be mediated through the effects of competition on the host plant. A reduction in the photosynthetic rate due to shading by neighbours may have been responsible (Bååth and Hayman 1984); this may not be significant in the field if pastures are kept short by grazing.

Interspecific competition did not influence VAM colonisation in this project (§11.2.d.ii). Presumably, severe interspecific competition could result in a decrease in colonisation in the plant being outcompeted, due to a reduced rate of photosynthesis. Although, Fitter (1977) found increased VAM colonisation in rye grass when roots intermingled with those of *Holcus lanatus* L., even though *H. lanatus* was outcompeting the rye grass. It is possible that *H. lanatus* stimulated spore germination or mycelial growth (see Ocampo *et al.* 1980).

(viii) Seasonal Variation

Regular seasonal variation in VAM colonisation levels could be expected to occur due to variation in environmental factors such as temperature, light and water. Colonisation of the wheat crops was highly seasonal as colonisation was determined by the phenology of these annual crops. However, in the dairy pastures there was no evidence of regular seasonal fluctuations in VAM colonisation. The lack of disturbance, relatively mild winters, irrigation over summer and perennial nature of the pasture all reduce the likelihood of obvious seasonal variation in VAM colonisation (§12.2.a). A lack of seasonal change in VAM colonisation has also been noted for other grassland communities in Australia and overseas (Sanders and Fitter 1992; Scheltema *et al.* 1987; Stürmer and Bellei 1994) and for forest communities in Western Australia (Brundrett and Abbott 1994).

(ix) Other Farm Management Practices

The effects of other farm management practices on VAM colonisation — such as soil disturbance, and addition of fungicides and herbicides — were not determined experimentally in this project. However, the results from the field surveys, discussed below, indicate that they did not play a major role in determining the levels of VAM colonisation on any of the farms.

Soil disturbance, as may occur through tillage, may reduce VAM colonisation by disrupting the hyphal network (Evans and Miller 1988; Evans and Miller 1990; Jasper *et al.* 1989). In wheat crops on the alternative mixed farms, initial VAM colonisation was rapid, with the maximum colonisation level of around 60% of root length being reached by tillering (70 days after sowing). Thus, although these farms all used traditional tillage, it seems unlikely that tillage markedly reduced the colonisation rate. VAM inoculum levels may have accumulated sufficiently under the annual pasture that preceded the crops such that tillage did not reduce the inoculum potential of the soil (Jasper *et al.* 1991); this may not be the case for second year crops.

Fungicides were not applied in large quantities on any farms sampled during this project. The conventional mixed farms applied seed dressings, however Ryan (1992; Fig. 5.18) and Spokes *et al.* (1989) found these did not affect VAM colonisation levels. Herbicides were only applied in significant quantities on the mixed farms. There are contrasting reports about the effects of herbicides on VAM colonisation (Ocampo and Barea 1985; Pope and Holt 1981; Tommerup and Briggs 1981). No general statements can be made, as the effects apparently need to be assessed individually for each herbicide, host plant and, perhaps, soil type. The results of Ryan (1992; Fig. 5.18) suggested herbicides as unlikely to affect VAM colonisation levels on the mixed farms sampled in this project, although they could still have affected the functioning of the fungi (Dehn *et al.* 1990; Kough *et al.* 1987).

(x) The Scale at which Factors Influence VAM Colonisation Levels

During this project, data were collected and analysed at a number of different scales ranging from individual plant, to sampling site, paddock and farming system. Relationships which were apparent at one scale were not always apparent at other scales, giving clues about the nature of the factors responsible for variation in VAM colonisation levels. Figures 12.1 to 12.3 compare regressions using data from individual sites or pots — leverage estimates were used to adjust for the effects of farm, location or treatment — with simple regressions using paddock or treatment means.

Figure 12.1 presents the relationship between VAM (%) and leaf P in wheat at tillering in 1993. Although there was a broad range of VAM colonisation levels and leaf P concentrations present at the individual sites, there was no significant correlation. However, the paddock averages exhibited a strong negative correlation (see also Tables 5.8 and 5.9); the negative influence of P was confirmed in glasshouse trials (Chapter 7). Variations in the distribution of VAM inoculum and the distribution of soil extractable P may have been disrupting the relationship between VAM colonisation and P at the finer scale. The relationship between shoot P and VAM (%) on the dairy farms was also stronger at the paddock level (Figs. 10.3.b and 10.7.a), however, the correlation at the individual site level was improved when root P was used (Fig. 10.7.c). Thus the variation at the site level in the wheat crops may also have reflected slight differences in the degree of allocation of P from roots to shoots, due to slight variations in the age of the youngest two fully-emerged leaves or the effects of pathogens.

Figure 12.2. presents the relationship between VAM (%) in clover and grasses, either rye grass or paspalum, at the individual site scale for two conventional/biodynamic farm pairs and as paddock means from 20 dairy farms. Similar relationships were present at each scale, although more variation was present at the site level. The sample of each species at each site was combined from 2-3 plants taken from a section of pasture 200 x 100 mm x 150 mm deep (§3.2). The roots of both species were generally intertwined. Again, the greater degree of variation at the site level would presumably be due to individual plant factors such as root age, degree of interspecific competition and disease. The stronger relationships at the site level on the dairy farms, compared to the crops, may be due to the perennial nature of the pasture resulting in both rapid colonisation of all new roots and a more constant relationship between root and shoot P concentrations (see §12.2.a).

Figure 12.3. presents the relationship between VAM (%) and shoot P in clover grown in two soils in a glasshouse trial under four fertiliser treatments (§11.1). A negative relationship was present at both the individual pot level (two plants combined) and the treatment level, however, the relationship was stronger at the treatment level. This again suggests that factors were interfering with the relationship between VAM and P at the individual plant or pot scale. As the soil in the trial was sieved and mixed,

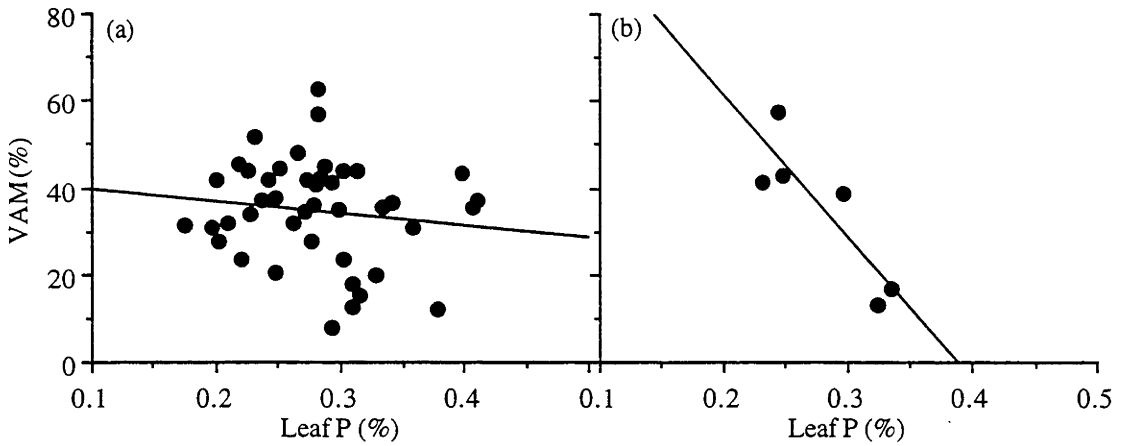


Figure 12.1. Relationship between the percentage of root length colonised by VAM fungi and the concentration of P in the youngest two fully-emerged leaves from 15 sites in six first year wheat paddocks in 1993 (§5.3.e.ii) using a) individual site data standardised by paddock means (r^2=0.02) and b) paddock averages (r^2=0.75).

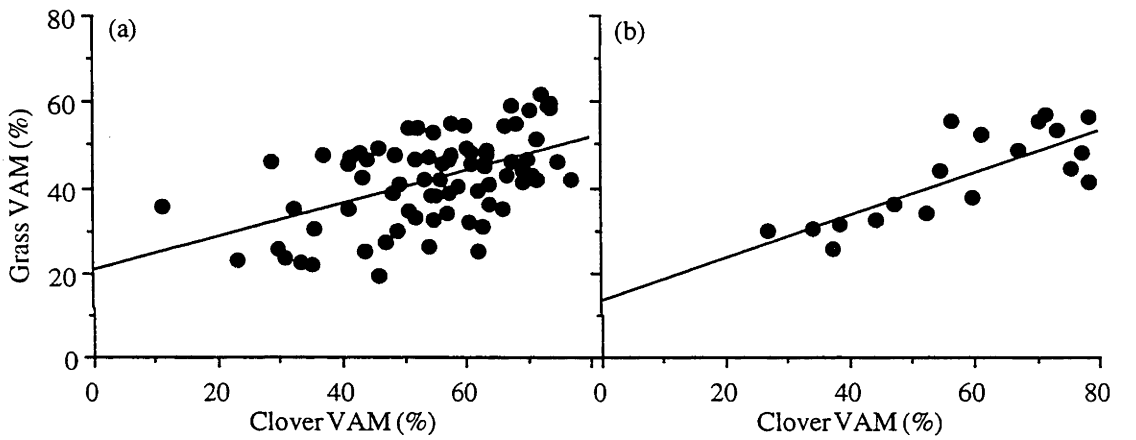


Figure 12.2. Relationship between the percentage of root length colonised by VAM fungi in grasses and clover on NE Victorian dairy farms in March 1993 (§10.3) using a) results from 20 sites in four paddocks standardised by paddock means (r^2=0.28) and b) paddock averages from 20 farms (r^2=0.64).

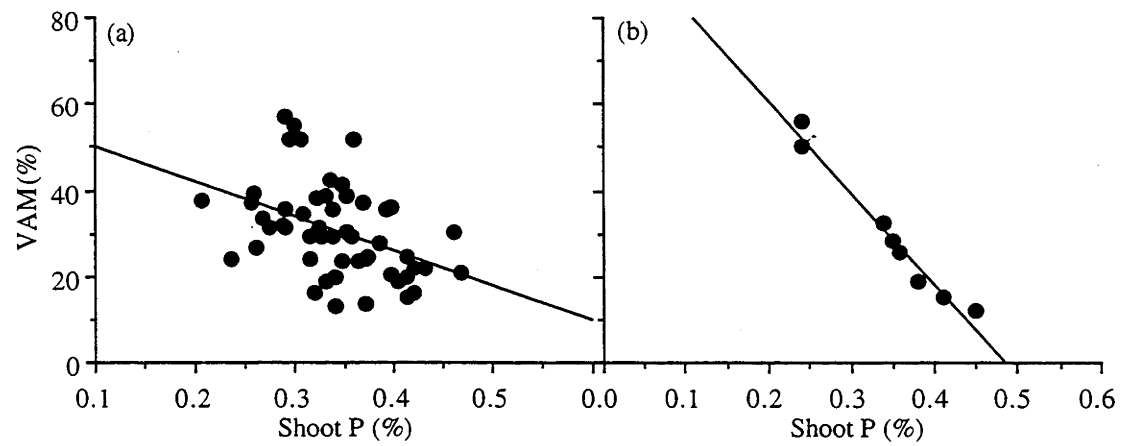


Figure 12.3. Relationship between the percentage of root length colonised by VAM fungi and the concentration of P in whole shoots of seven clover plants from each of eight treatments in a glasshouse trial (§11.1) using a) individual plant data standardised by treatment means (r^2=0.18) and b) treatment averages (r^2=0.97).

variation in inoculum or soil extractable P is unlikely to be the cause of this variation, however differences in the environment in individual pots, due to placement in the glasshouse or watering (§3.7), could be responsible. Plants were an identical age, appeared disease-free and were of the same variety grown from the same seed source. However, variation could be due to the plant P-VAM relationship not being tightly controlled due to the absence of a tight mutualistic relationship between the plant and the fungi (see §12.1.c.iv). This could be exacerbated in these young plants growing under optimal conditions, compared to the field results in Figure 12.2, due to rapid root growth; this could also increase the variation associated with subsampling of root systems.

However, the strong relationship in Figure 12.3.b. indicates that seven replicates were adequate to virtually eliminate the effects of this variation on the relationship between VAM (%) and shoot P. It also indicates that, on average, there was a very tight relationship between root P — which presumably controls VAM colonisation levels (§12.1.a.ii) — and shoot P in these young plants. Overall, Figures 12.1-12.3 illustrate the importance of independent replicated sampling in both the field and the glasshouse.

Repeated sampling from paddocks to investigate temporal variation in VAM colonisation levels was also used in this project. The results indicated that to assess differences in VAM colonisation between annual crops it is necessary to sample soon after seedling emergence and then 3-4 additional times over the season, as differences in colonisation levels may initially be very large, but decrease as the plants approach senescence (Fig. 5.1). In irrigated perennial pasture, there was no evidence of seasonal trends and thus a one-off sampling should generally be adequate; this may not be the case for non-irrigated annual pasture, where VAM (%) may be more variable due to plants recolonising each year and greater fluctuations in soil water.

12.1.b. What Influence Does Colonisation by VAM Fungi have on Plant Growth?

This section considers the effects of colonisation by VAM fungi on the dominant plants growing in the mixed farm and dairy farm systems using results from the glasshouse trials presented in Chapters 7 and 11. Factors which may affect the relevance of these results to field conditions are then discussed and conclusions drawn about how to design glasshouse trials that will produce results applicable to the field situation.

(i) VAM Fungi and Plant Growth Under Glasshouse Conditions

Shoot Biomass, Root Biomass and the Root-Shoot Ratio

Table 12.1 summarises the effects of VAM colonisation on the root biomass, shoot biomass and root-shoot ratio of the five main plant species examined in this project. VAM fungi did not significantly affect the growth of wheat in mixed farm soil, even

though P limited wheat growth. The effects of VAM fungi on wheat growth have been reported to vary from negative to strongly positive (Buwalda *et al.* 1985a; Khan 1975; Mohammad *et al.* 1995). Along with the results from the first glasshouse trial — where VAM fungi had a parasitic effect on wheat growth apparently due to low light levels (§7.2) — this suggests that the relationship between VAM fungi and wheat is finely balanced, with changes in environmental or soil conditions affecting the costs and benefits of the relationship; see also Manske (1989).

Table 12.1. Summary of the effects of VAM colonisation on the root biomass, shoot biomass and root-shoot ratio of plants grown in soil from the mixed farms (3.5 $\mu g\ g^{-1}$ Olsen extractable P) or the dairy farms (43 $\mu g\ g^{-1}$ Olsen extractable P). Root and shoot biomass are given as the percentage difference from non-VAM controls and the root-shoot ratio is indicated as having increased (+) or decreased (-) relative to the non-VAM controls.

	Soil	Shoot	Root	Root-shoot ratio	Thesis section
Wheat	low P	+ 2%	0%	-	7.3†
Rye grass	high P	- 3%	+ 18% *	+ *	11.2
Paspalum	high P	+ 7%	+ 16% *	+	11.2
White clover	high P	+ 18% *	+ 35% *	+ *	11.2
Subterranean clover	low P	+ 60% *	+ 34% *	- *	7.3

* significantly different from non-VAM controls at $p<0.05$.
† field VAM treatment excluded as it was associated with insufficient colonisation.

In dairy farm soil, VAM colonisation did not increase rye grass shoot growth, but increased root growth by 18%, while paspalum root and shoot growth were both slightly increased. Rye grass generally does not respond positively to VAM colonisation, even at low soil P (Buwalda and Goh 1982; Crush 1995; Fitter 1977; Schweiger *et al.* 1995; see §12.1.c).

Shoot growth of white clover in the dairy farm soil was increased by 18% by VAM colonisation, while growth of subterranean clover in the lower-P mixed farm soil was increased by 60% by VAM colonisation. Clovers are generally highly dependent on VAM fungi, with large increases in growth occurring when colonised in low P situations (Puppi and Bras 1989; Schweiger *et al.* 1995). The results in Table 12.1 are consistent with the concept of VAM dependency (§1.3.d), with dependence being greater for legumes/coarser rooted species and in the lower-P soil (see also §12.1.c).

Root-shoot ratios generally decrease in response to VAM colonisation (Koide 1991; Smith and Read 1997), due to VAM hyphae essentially substituting for roots (Jakobsen 1996) and the consequences of an increased P supply for plant growth (Ericsson 1995; Wilson 1988b). However, while VAM colonisation decreased the root-

shoot ratio of plants in the mixed farm soil, it increased the root-shoot ratio of the three species grown in the dairy farm soils.

Plants grown in the dairy soil were not significantly P-limited (§11.2). VAM colonisation may trigger a plant physiological response resulting in more photosynthate being allocated to the roots and being used for both fungal and root growth (Snellgrove *et al.* 1982). When VAM fungi contribute towards a nutrient deficiency being overcome, shoot growth increases proportionally more than root growth and the root-shoot ratio is decreased. However, when nutrients are not limiting, the enhanced root growth may not result in proportionally greater shoot growth and the root-shoot ratio may remain constant or even increase (Fig. 12.4).

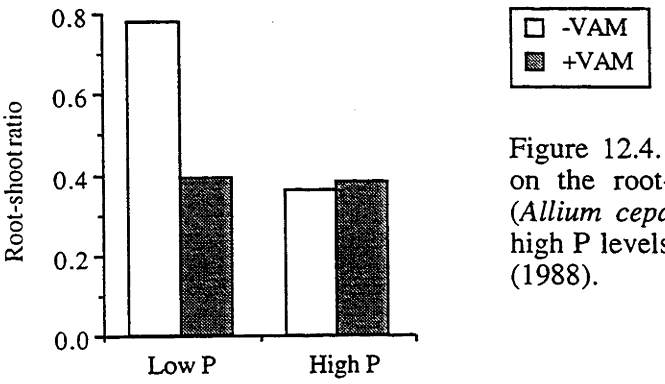


Figure 12.4. The effect of VAM fungi on the root-shoot ratio of leek plants (*Allium cepa* L.) grown under low and high P levels. Data from Son and Smith (1988).

Other Effects on Plant Growth

VAM fungi may influence plant growth other than through effects on biomass and the root-shoot ratio. For instance, VAM fungi may affect the ratio of biomass in stems to biomass in leaves (Koide 1985), factors associated with reproduction — such as the time to initiate flowering, seed numbers and seed nutrient concentrations (Lu and Koide 1994; Sanders and Koide 1994) — and may promote a prostrate shoot morphology (Wallace 1981). This project generally did not examine such factors, although in the wheat grown in the glasshouse trial in section 7.3, the weight of the heads and the total shoot weight responded in the same manner to VAM colonisation (Fig. 7.4).

The differences between VAM colonised plants and non-VAM plants may vary over time. For instance, Bloss and Pfeiffer (1984) found survival of transplanted guayule seedlings (*Parthenium argentatum* A. Gray) was much higher for VAM inoculated seedlings; although this interpretation must be treated with caution, as the inoculated seedlings were twice as big as non-VAM seedlings at transplanting. Cade-Menun (1991) reported that wheat seedlings were more dependent on VAM fungi than mature plants. In the glasshouse trial using dairy soil (§11.2) the differences in total

biomass of VAM colonised and non-VAM clover plants increased over time (Fig. 11.11).

It is also possible that VAM fungi influenced the concentration of nutrients, other than P and N, in the plants examined in this project (Marschner and Dell 1994). However, as the glasshouse trials had basal nutrients applied to ensure that only P, or P and Zn, limited plant growth, further experiments are necessary to clarify this point.

There is increasing evidence for VAM colonisation conferring disease resistance on the host plant, including host plants not reliant on VAM fungi for nutrient uptake (Watkinson *et al.* 1996; Newsham *et al.* 1995; see §12.1.c.iv). It is possible that VAM fungi reduce the incidence of disease on both the mixed and dairy farms examined in this project (see also Thompson and Wildermuth 1989). In particular, the rapid initial colonisation of alternative crops and high levels of colonisation present in alternative crops and pastures may make VAM effective at blocking colonisation of roots by pathogens. This was not examined during this project and is a topic meriting further research.

(ii) Are Glasshouse Trials Applicable to Field Conditions?

This section discusses the major design factors that potentially reduce the applicability to field conditions of predictions from glasshouse trials which assess the effects of VAM colonisation on plant growth. Each subsection concludes with suggestions for addressing the problems it raises and these are summarised at the end of the section. Many of the factors which may be responsible for the minimal response to VAM colonisation which has been found under field conditions are also discussed (Fitter 1985). How to best design field trials to accurately assess VAM functioning is not addressed here and the reader is referred to Jakobsen (1992) for a general overview

<u>The Soil Environment</u>

To reduce variation between replicates within each treatment, all soil used in glasshouse trials in this project was sieved and thoroughly mixed before pots were filled. This would have also reduced the natural heterogeneity of P distribution in the soil and may have thereby affected the role of VAM hyphae in accessing P for the plant. The variation in factors, such as P, down the soil profile is also removed by sieving. Sieving destroys the hyphal network which, in the field, may increase the inoculum potential of the soil and — through hyphae from newly colonised seedlings linking into the network — increase the ability of VAM fungi to take up P (Evans and Miller 1990; Miller and McGonigle 1992). This may only be important in the dairy soils, as the soils on the mixed farms were cultivated before sowing, perhaps reducing the effectiveness of VAM fungi in comparison to a crop sown using minimum tillage (Kabir *et al.* 1997).

Using intact soil cores in glasshouse trials (Evans and Miller 1990) would reduce these problems, but could increase the variability of results.

Soil nutrient concentrations may also differ from the field, particularly if the field soil contains a high density of live roots and VAM hyphae — as was the case on the dairy farms — resulting in strong competition for any P released chemically or biologically; see Newman and Eason (1989). In a glasshouse trial using field soil, this competition would be absent and along with the decay of dead organic matter in the soil, this may result in P becoming relatively readily available to plants. This may have occurred in the glasshouse trial presented in section 11.2.

Use of fungicides or soil sterilisation to create non-VAM controls affects the other soil micro-organisms present. There is increasing evidence that other soil micro-organisms will influence the effects of VAM fungi on plant growth. For instance, Hetrick *et al.* (1988) found that soil micro-organisms could stimulate plant growth to a similar degree to VAM fungi and when VAM fungi and soil micro-organisms were both present, the micro-organisms greatly suppressed VAM activity. Toro *et al.* (1997) reported that addition of P-solubilising bacteria increased VAM colonisation and improved the uptake of P from rock phosphate by VAM colonised plants in a low P soil. Thus, estimates of the benefits of VAM fungi to plant growth obtained in sterilised soil, may over- or under-estimate the contribution of VAM fungi relative to field conditions (see also Koide and Li 1989).

This problem is generally addressed, including in this project, through the addition of non-sterile soil sievings to all treatments (§3.7). However, even if micro-organisms are reintroduced using sievings, the populations are unlikely to be identical to those in the field, as micro-organism populations in the non-VAM pots may not be restored to the same level and composition as in the pots inoculated with non-sterile soil. Glasshouse temperature and watering regimes will also result in soil micro-organism populations differing from those in the field.

Glasshouse trials using sterilised soil may lack the pathogens present in the field, the protection from which may be the primary benefit for plant growth from VAM colonisation (Newsham *et al.* 1995). Alternatively the accidental introduction of diseases through contaminated VAM inoculum, combined with favourable growth conditions in the glasshouse, may confound results through leading to much higher levels of disease than would occur in the field. Levels of disease may also vary significantly between pots due to slight differences in environmental conditions.

Grazing of hyphae by insects, such as collembola, may greatly affect the functioning of VAM fungi in the field (Warnock *et al.* 1982). Smith and Read (1997) compiled a table comparing the length of VAM hyphae in glasshouse trials and in field soils, however, the number of studies using field soils was insufficient to state whether hyphal lengths are consistently lower.

Environmental Conditions

Light, temperature and water all have the potential to affect the degree of VAM colonisation and the effects of VAM colonisation on plant growth. For instance, low and more diffuse light conditions in glasshouses have the potential to reduce the growth response to VAM inoculation through lowering the plant photosynthetic rate (§7.2; Bethlenfalvay and Pacovsky 1983; Smith and Gianinazzi-Pearson 1990).

Buwalda *et al.* (1985b) found VAM colonisation in winter cereals did not increase when air temperatures were <5°C. Smith and Bowen (1979) found that the number of VAM entry points to roots increased as soil temperature increased from 12-25°C, while Stutz and Martin (1996) reported significant decreases in VAM colonisation at high air temperatures (42/32°C day/night). Moreover, temperature is likely to influence which species of VAM fungi colonise roots. Braunberger *et al.* (1997) found *Glomus* spp. predominated in warm soil (31°C), while *Acaulospora* spp., *Scutellospora* spp. and fine endophyte were most abundant in cooler soil (18°C). One factor responsible for such differences may be variation in the temperature limits for spore germination (Tommerup 1983).

Most glasshouse studies examining VAM fungi and water stress have applied a relatively mild level of water stress, such as rewatering after a short period of leaf wilting (Allen and Boosalis 1983; Ellis *et al.* 1985; Simpson and Daft 1990) or allowing soil matric potential to decrease to -1 MPa (Bethlenfalvay *et al.* 1988). Unlike the field results from 1994 (Chapter 8), these studies do not find a negative effect on VAM colonisation (Bethlenfalvay *et al.* 1988; Simpson and Daft 1990; Sylvia *et al.* 1993). Presumably the level of stress experienced by the host plant was not sufficiently high to significantly reduce the photosynthate available to the fungi. In addition, most studies have involved plants being well-watered, and consequently highly colonised by VAM fungi, before the imposition of the drought treatment (Allen and Boosalis 1983; Auge *et al.* 1986); this may not be the case for non-irrigated field crops.

Thus light, temperature and watering regimes should be chosen to be as close as possible to the conditions in the location where the results will be applied. Researchers should also be aware of the potential of small pots in glasshouse trials to increase the variability of soil temperature and water, as the buffering capacity of a large soil volume is absent. This problem could be minimised through keeping pots in constant temperature water baths, weighing pots to allow watering to a specific soil matric potential regularly, use of large pots and having a buffer row of pots around the edge of the trial.

Plant Characteristics

Plant community characteristics likely to differ between glasshouse and field conditions are root density, plant P requirements and the degree of both intraspecific and interspecific competition between roots. The benefits to the host plant from VAM colonisation may decrease as root density increases or as the soil available to a given plant decreases (Allsopp and Stock 1992; Bååth and Hayman 1984; Koide 1991; Fig. 11.15.b). Koide (1991) grew *Abutilon theophrasti* Medic. at four densities, with and without VAM fungi. Non-VAM plants at higher densities had a far higher level of P acquisition than those at low densities, while plants colonised by VAM fungi at higher densities had only a slightly higher level of P acquisition than those at low densities (Fig. 12.5). Koide (1991) suggested that the VAM colonised plants at the low densities were closer to depleting the available soil volume of P due to the added length of VAM hyphae; a combined density for roots and hyphae, excluding root hairs, was calculated to be 215 cm cm^{-3}.

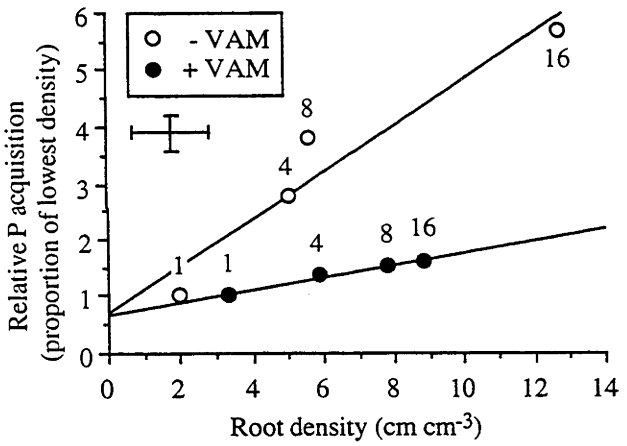


Figure 12.5. Relationship between relative P acquisition (above-ground) and root density for *Abutilon theophrasti* Medic. grown with and without VAM fungi. Error bars are 95% confidence intervals. Numerals near symbols indicate the number of plants in each pot. Adapted from Koide (1991).

Table 12.2. presents the estimated density of roots, root hairs and VAM hyphae for a conventional and organic wheat crop at anthesis and, based on measures by Cock (1991), conventional and biodynamic dairy pastures. Active (white) root length is given for both systems, as it is likely to be a more accurate estimate of the length of root available for nutrient absorption, due to nutrient uptake declining in older roots (Newman and Andrews 1973). Caution must be used when applying the results from glasshouse density experiments, where all roots are young, to field situations. For instance, Jasper *et al.* (1991) classified 50% of roots under an annual pasture and >90% of roots under a Eucalypt forest and a heathland as 'old'.

Table 12.2. Approximate length (cm cm^{-3} of soil) of roots, root hairs and VAM hyphae under a conventional and organic wheat crop at anthesis (0-50 mm)(1) and under conventional and biodynamic irrigated dairy pasture in late spring (100-200 mm)(2). Dairy pasture measures are taken from Cock's (1991) study of a conventional/biodynamic farm pair in the Murray River Valley and figures are for the top 100-200 mm of soil, as the root density in the top 100 mm was extremely high and not measured. Active roots were assumed to be the white roots in both this project (§3.4.a) and by Cock (1991).

	Root type	Roots	Root hairs(3)	VAM hyphae(4)
Wheat on mixed farms at anthesis (0-50 mm)(1)				
Conventional	active	7	107	301
Organic	active	17	260	1020
Dairy pasture (100-200 mm)(2)				
Conventional	active	10	122	200
	total	135	1652	2700
Biodynamic	active	14	171	700
	total	150	1836	7500

(1) see Figures 5.1 and 5.5 for root length and VAM colonisation levels.
(2) 80% of roots were assumed to be from grasses; average VAM colonisation was assumed to be 20% of root length for the conventional pasture and 50% for the biodynamic pasture.
(3) estimated as 15.3 cm cm^{-1} of root length for wheat and grasses and 0 for clover (Föhse *et al.* 1991).
(4) estimated as 100 cm cm^{-1} of VAM colonised root but, may be up to an order of magnitude higher (Abbott and Robson 1985b; Abbott *et al.* 1992; Miller and Jastrow 1992a; Tisdall and Oades 1979); Gatehouse (1995) found 15 m g^{-1} of hyphae under an organic wheat crop at Ardlethan at anthesis in 1995, which assuming 1993 root lengths (Fig. 5.8), is around 200 cm of hyphae cm^{-1} of colonised root.

In the wheat crops, root density in the top 50 mm of soil — where root density is greatest (Fig. 5.8) — ranged from 7-17 cm cm^{-3}. Newman and Andrews (1973) used various pot sizes to manipulate wheat root density and found no competition between roots for P at total root densities up to 40 cm cm^{-3} in a very low P soil; as they used diluted non-sterile field soil, it is possible that the plants were colonised by VAM fungi. Thus it seems likely — depending on the ratio of active to total roots in the field — that in the wheat crops sampled during this project, root densities were not high enough for roots to be competing for P and reducing any benefits from VAM colonisation. Although, the combination of root length and hyphae was 1037 cm cm^{-3} in the organic crop, which is far higher than the 215 cm cm^{-3} calculated by Koide (1991) as being close to allowing *A. theophrasti* to deplete the soil of P. Similar conclusions can be drawn for the dairy pastures. Overall, the lack of literature on this topic makes it impossible to draw definite conclusions about whether the root densities on the farms sampled during this project were high enough to reduce the effects of VAM colonisation.

In the field, plants generally grow more slowly than in the glasshouse and do not require P as rapidly. Thus, natural diffusion of P back into depletion zones around roots may be more important, and VAM hyphae less important, in plant P uptake. Fitter and Merryweather (1992) summarised P inflow rates from a number of studies, to show that inflows under natural conditions were generally an order of magnitude less than in the glasshouse; however it was not clear whether this would also be the case for agricultural crops. In addition, growth of plants in the field may be limited by competition, pathogens or environmental factors other than P (Newsham *et al.* 1995) and may be on a seasonal cycle not present in a glasshouse resulting in VAM colonisation only being important at certain times of year (Dunne and Fitter 1989).

In pastures and natural habitats, plants compete with neighbours of different species for resources. In glasshouse trials, colonisation by VAM fungi has been found to influence the outcome of interspecific competition in favour of more mycotrophic species (Crush 1995; Fitter 1977; Grime *et al.* 1987; Hall 1978); although this effect is reduced if P is abundant (Crush 1995; Hall 1978; see Fig. 12.6). Thus the influence of VAM fungi in pastures, and similar species diverse communities, may include mediating the outcome of interspecific competition. Although the reduced effect of competition from grasses on clover when regular defoliation was occurring (Fig. 11.10.a) indicates that if there are constant high levels of grazing, the importance of VAM fungi in mediating competition outcomes may be much reduced. Alternatively, continual removal of shoots may result in reduced root systems and a greater role for VAM fungi in plant nutrient uptake.

Thus, when conducting glasshouse trials to assess the effects of VAM fungi on plant growth, it could be important to include a range of plant densities and consider the effects of interspecific competition if results are to be relevant to field conditions; an appropriate defoliation/grazing treatment may also be necessary. The duration of trials also becomes important. This is because in pots, a range of initial plant numbers will reach the same final biomass and thus measurement of relative growth rate, as well as final biomass, could be beneficial. In addition, initial growth may not be the most important factor in measuring the fitness of long-lived plants (Allen *et al.* 1992). Factors such as survival and reproduction should also be measured whenever possible.

The Species of VAM Fungi

Glasshouse trials often use single species of VAM fungi, often not isolated from — or even present in — the location to which the results are to be applied. Field sites commonly contain a community of VAM fungi consisting of 3-10 species from 2-3 genera (Clapp *et al.* 1995; Douds *et al.* 1995; Gemma *et al.* 1989; Hayman and Stovold 1979; Hendrix *et al.* 1995; Johnson 1993; Pringle and Bever 1996) and plants may exhibit a greater growth increase when a number of species of VAM fungi are present

in their root systems (Edathil *et al.* 1996). VAM species and even isolates of species may differ greatly in their effects on plant growth, soil aggregation and soil microbial populations (Abbott and Robson 1985b; Allen and Boosalis 1983; Gavito and Varela 1995; Graham *et al.* 1982; Schreiner *et al.* 1997).

As it can be difficult to identify the species present in the field and some of these may be difficult to establish in pure cultures (J. Morton, pers. comm.), inoculation with field soil — as occurred in this project — is the best way to mimic the field VAM populations. However, even then it is unlikely that the relative levels of colonisation and functioning would entirely resemble those in the field, due to the different environmental conditions in the glasshouse.

Glasshouse Trials : Conclusions

While controlled glasshouse trials using single fungal isolates inoculated into sieved soils are necessary to find out the potential physiological interactions between VAM fungi and host plants, glasshouse trials should also be conducted with the aim of generating results that, as closely as possible, mimic the field situation. As discussed above, such glasshouse trials could include the following attributes:

- use of undiluted field soil, maybe as intact cores;
- use of non-sterile field soil as inoculum;
- equalisation of soil micro-organism populations through use of suitable filtrate;
- appropriate plant densities;
- mimicking of field light, temperature and water regimes as closely as possible;
- use of relatively large, deep pots;
- inclusion of appropriate interspecific competition treatments;
- growth of plants through their life-cycle for annuals and as long as feasible for perennials;
- measurement of other components of growth besides biomass;
- continual data collection to construct a time series and allow calculation of relative growth rates.

In this project the contribution of VAM fungi to plant growth was assessed using glasshouse trials, due to the problems associated with creating non-VAM controls in the field (§1.5), a lack of resources for sterilising soil in the field and soil sterilisation not being permitted under alternative farming standards (§2.3). Through using many of the above techniques, the glasshouse trials in this project were designed to be as relevant to field conditions as possible.

12.1.c. A Mutualistic or Parasitic Relationship?

This section briefly presents current theories about the evolution of the mycorrhizal symbiosis, which are then used — along with a discussion of the circumstances under which VAM fungi act as parasites or mutualists — to discuss two hypotheses about the form of the VAM fungi-host plant relationship.

(i) Evolution of the VAM Fungi - Host Plant Relationship

The earliest known land plants did not possess true roots, but fossils have shown infections by fungi which formed arbuscules, vesicles and intercellular hyphae similar to modern VAM fungi (Pirozynski and Dalpé 1989). It has been suggested that the limiting nutrient when the vascular plants first appeared was P (Nicolson 1975). Thus the colonisation of land by plants may have been made possible by VAM fungi enhancing host plant P uptake, while in return, they exploited a reliable source of energy; host plant photosynthate. Indeed, plants from nutrient-poor habitats resembling those present when plants first invaded the land — for example, sand dunes and volcanic debris — are commonly mycotrophic (Allen 1991; Gemma and Koske 1992), as are those plants with coarse root systems similar to those of the first plants.

For instance, bluebells, *Hycaninthoides non-scripta* (L.) Chouard ex Rothm., have coarse unbranched roots which, when non-mycorrhizal, are poorly able to forage for P. In a field study, Merryweather and Fitter (1995a; 1995b) strongly correlated the level of root colonisation by VAM fungi with inflow of P. An order of primitive living angiosperms, Magnoliales, have a high incidence of species which associate with VAM fungi (Baylis 1975). These plants tend to have coarse roots, > 0.5 mm diameter, with few root hairs and slow root growth and they tend, along with plants with similar roots, to be highly dependent on VAM fungi (Baylis 1975).

Thus, the ability to form VA mycorrhizas appears to be an ancestral condition and it is retained in the majority of plant taxa. Other forms of mycorrhizas, such as ericoid and orchid mycorrhizas, along with nonmycotrophy — which appears to have arisen numerous times — are probably derived (Allen 1991). However, plants have continued to evolve other attributes since invading land, including the development of more finely branched root systems, and it is conceivable that the role of VAM fungi has also evolved.

(ii) When do VAM Fungi Enhance Plant Growth

Many experiments have shown the importance of VAM fungi in enhancing plant P uptake. For example, Hall (1978) found that for white clover, grown in both a monoculture and a polyculture, VAM colonisation significantly increased plant growth at low levels of P. However, the costs of supporting VAM colonisation began to outweigh the benefits as P levels increased (Fig. 12.6.); such growth depressions have

often been found to appear as P availability increases (Crush 1995; Peng *et al.* 1993). Figure 12.7 presents a similar example from this project for white clover grown in soil from a biodynamic dairy farm (§11.2). Although the interaction between P and VAM colonisation was not significant, addition of VAM fungi significantly increased growth of plants at the lower P level, but had no effect on growth of plants at the higher P level. When rye grass was grown with the same treatments as the white clover in Figure 12.6, it exhibited little response to VAM inoculation or P addition (Fig. 12.8).

Two conclusions can be drawn from these examples. First, VAM colonisation favours P uptake in plant species with relatively coarse root systems — such as clover — when soil P levels are low. This supports the hypothesis that the primary benefit to plant growth from VAM colonisation was originally enhanced P uptake. Secondly, the finer and more branched root system and abundant root hairs of plants such as rye grass are better able to absorb P than the roots of clover and, therefore, they do not benefit from VAM colonisation.

However two questions remain. While this project has shown clearly that abundant P results in decreased VAM colonisation (§12.1.a.ii), the results in Figure 12.6. show that plants are not able to reduce sufficiently VAM colonisation in high P situations to avoid a negative impact on growth. VAM fungi can be a substantial drain on host photosynthate; Jakobsen and Rosendahl (1990) found up to 20% of photoassimilated ^{14}C in cucumber seedlings (*Cucmis sativus* L.) being used in the biomass and respiration of VAM fungi. Such experiments were conducted, however, on young, highly colonised (95%) plants grown under P-limiting, but otherwise optimal, conditions and older plants in the field may have a slower growth rate and lower, less active, colonisation. It is also curious that plants, such as rye grass, which are not dependent on VAM fungi for P uptake — even under low P conditions (Figs. 12.8 and 5.1) — may still be quite highly colonised.

(iii) Results from Field Trials

Experiments involving the transplantation of VAM inoculated and non-VAM seedlings into the field have produced results not readily explicable in terms of P availability. For instance, Hayman and Mosse (1979) transplanted white clover seedlings into a low P soil in Wales. The seedlings were planted either in a monoculture or into slits cut in turf (polyculture) (Fig. 12.9). As in Figure 12.6, the clover grew best in the monoculture. However, in contrast to Figure 12.6, VAM colonisation enhanced clover growth most when P was most abundant. Rangeley *et al.* (1982) planted white clover seeds into a red brown earth in New Zealand (Fig. 12.10). Seeds were either not inoculated or inoculated with *Glomus mosseae* or *G. etunicatus*. While the trends were not significant, it is curious that colonisation by *Glomus mosseae* tended to increase shoot growth, even though the plants showed no response to P addition. These field

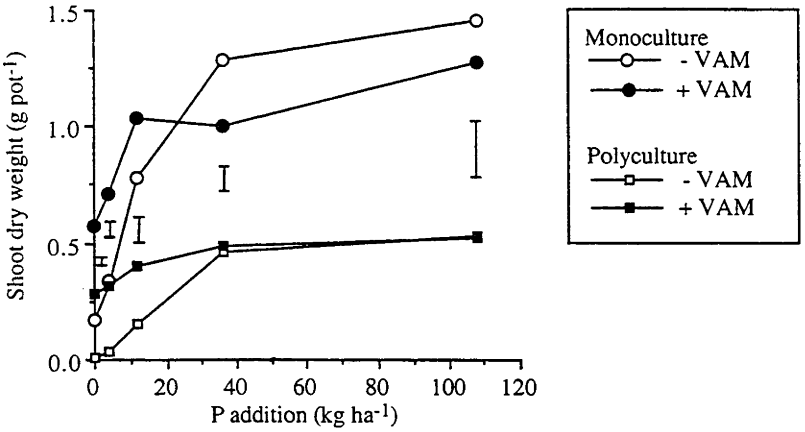


Figure 12.6. Shoot dry weight of white clover grown under five levels of P, with or without VAM fungi, and either in a monoculture (two clover plants pot^{-1}) or a polyculture (two clover and two rye grass plants pot^{-1}). Approximate LSDs at p=0.05 are given for each level of P. Data from Hall (1978).

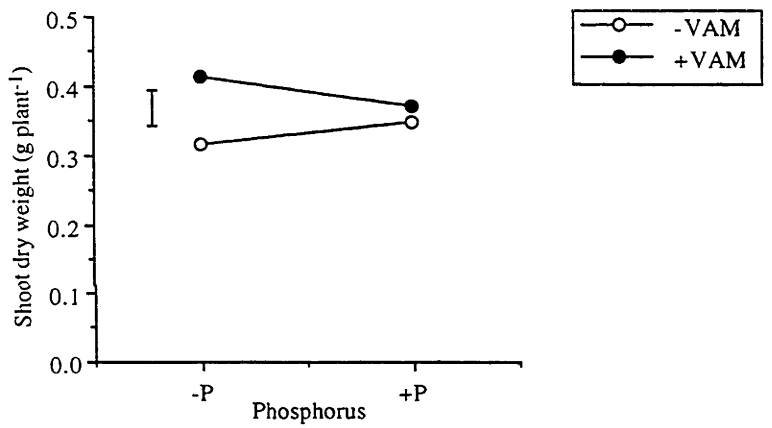


Figure 12.7. Estimated means and LSD at p=0.05 for the interaction between VAM inoculation and P addition for shoots of white clover grown in a glasshouse trial (§11.2). The interaction was not significant (p=0.07).

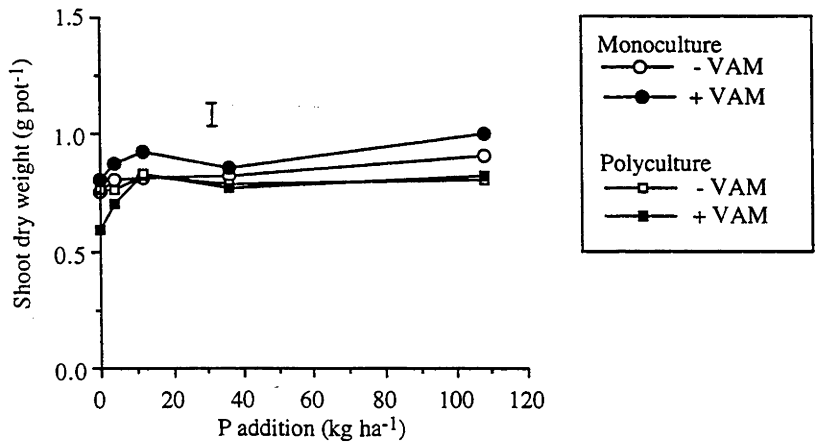


Figure 12.8. Shoot dry weight of rye grass grown under five levels of P, with or without VAM fungi, and either in a monoculture (two rye grass plants pot^{-1}) or a polyculture (two clover and two rye grass plants pot^{-1}). Approximate LSD at p=0.05 for all levels of P combined. Data from Hall (1978).

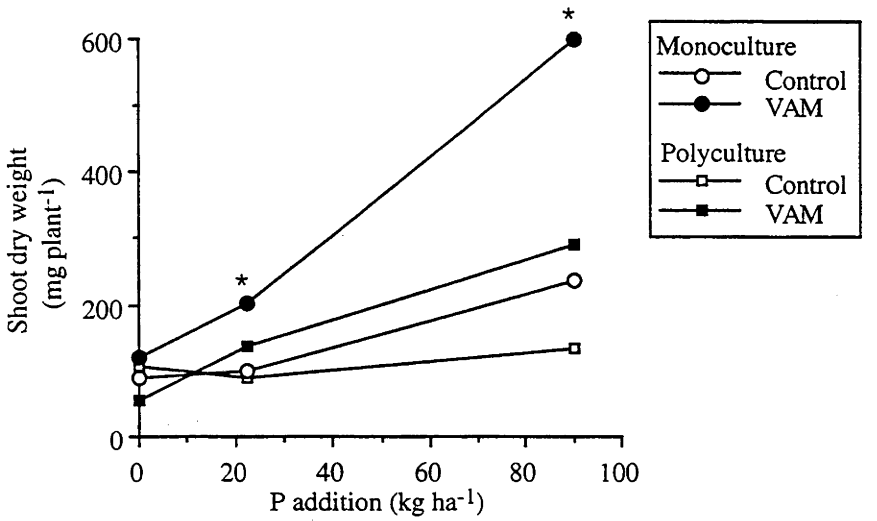


Figure 12.9. Shoot dry weight of white clover 12 weeks after being transplanted into the field as either non-VAM controls or seedlings inoculated with *Glomus mosseae*. Seedlings were planted in rows either into bare peat where the turf had been removed (monoculture) or into 2 cm slits cut into the turf (polyculture). Note that by week 12, all plants were colonised by VAM fungi, with the controls averaging 46% and the inoculated seedlings 74% of root length colonised. * significantly different from non-VAM plants under same treatments at $p<0.05$. Data from Hayman and Mosse (1979).

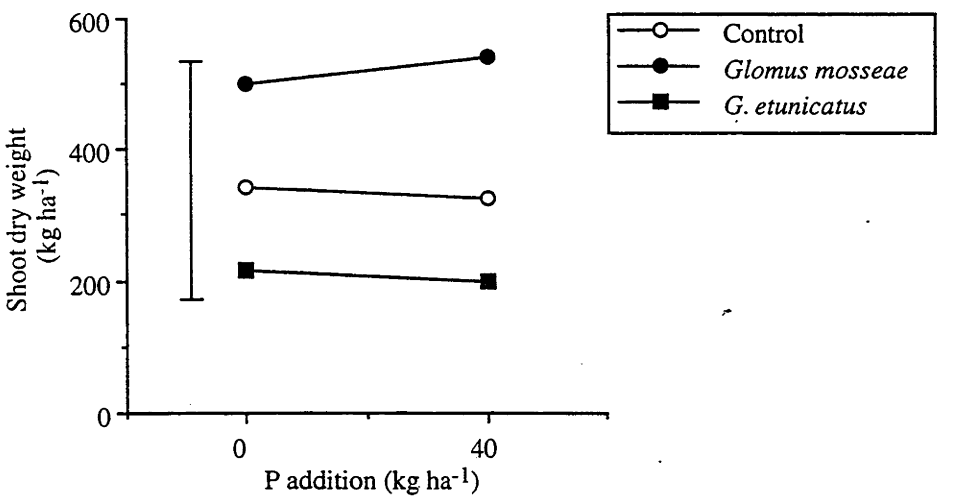


Figure 12.10. Shoot dry weight and LSD at $p<0.05$ of white clover 6 months after being sown into cultivated field soil, either without VAM inoculum or with inoculum consisting of root segments colonised by *Glomus mosseae* or *G. etunicatus*. Note that by the time of sampling, VAM colonisation levels in all treatments were similar. Data from Rangeley *et al.* (1982).

results suggest that the response of plants to VAM colonisation in the field, even strongly dependent plants like clover, is more complicated than the simple relationship with P shown in Figures 12.6 - 12.8; see also McGonigle and Fitter (1988).

The discussion in section 12.1.b.ii suggested that the results from glasshouse trials, the most common method for assessing the contribution of VAM fungi to plant growth, may not always accurately reflect the situation in the field. Unfortunately, field trials are difficult to conduct due to the problems associated with creating non-VAM controls (§1.5). Factors present in the field which could be modifying the P-VAM fungi-host plant relationship include the following (see also the discussion in §12.1.b.ii):

- interactions with other soil micro-organisms;
- grazing of hyphae by invertebrates;
- slow plant growth rates and reduced plant P requirements;
- plant and fungal growth being limited by factors other than P;
- high plant root densities;
- plants being linked to a hyphal network;
- grazing of plants reducing interspecific competition.

Two hypotheses have been proposed to explain the retention of VAM colonisation by species, and in field situations, where plant P uptake is not enhanced; I have termed these the disease hypothesis (see Newsham *et al.* 1995) and the periodic stress hypothesis (see Allen 1991).

(iv) The Disease Hypothesis

Recent experiments, reviewed by Watkinson *et al.* (1996), indicate that in some cases, the primary benefit to plants from VAM colonisation in the field may be protection from pathogens. For instance, Newsham *et al.* (1995) grew seedlings of the annual grass *Vulpia ciliata* (le Gall) Stace and Auquier in the field. VAM colonisation did not obviously affect plant P nutrition. Rather, as the percentage of root length colonised by VAM fungi increased, the percentage of root length infected by the pathogen *Fusarium oxysporum* deceased. VAM colonisation significantly increased plant growth only when *F. oxysporum* was present, presumably because *V. ciliata* has very fine roots which are relatively efficient at exploring the soil and absorbing nutrients. Thus, it appears likely that VAM fungi protect *V. ciliata* from the substantial reductions in fecundity (up to 50%) which can be caused by the pathogen.

Newsham *et al.* (1995) suggest that the persistence of VAM fungi in such species may be due to their ability to protect plants from pathogens. However, as the VAM fungi were not acting parasitically when the pathogen was absent, presumably the VAM symbiosis would not be actively selected against.

(v) The Periodic Stress Hypothesis

In natural ecosystems, the fitness of a plant reflects both its ability to survive to reproductive maturity, as well as its yield in a single growing season (Allen 1991) and, therefore, the ability of a plant to survive periods of stress will be important. Periodic stresses could involve a number of factors including water (Allen and Allen 1986; Fitter 1985), nutrients and, as discussed above, disease (Newsham *et al.* 1995). If VAM fungi allow a plant to survive through a period of stress, then the fungi may improve the fitness of the plant, even though there may be a constant carbon loss throughout the life of the plant (Allen 1991).

Consequently, comparisons of VAM-inoculated plants with non-VAM controls under optimal glasshouse conditions or in short term field trials may not find VAM colonisation to be beneficial because plants were not subjected to stress. Moreover, although size may generally be a good predictor of a plant's ability to survive and successfully reproduce, other factors may also be important; for instance seed P content (Derrick and Ryan 1998; Koide *et al.* 1988).

The periodic stress hypothesis is consistent with the tendency of plants to be colonised by a number of VAM species (Edathil *et al.* 1996 and §12.1.b.i) — variation in hyphal characteristics and functioning would allow a broader range of stresses to be alleviated (Allen 1991) — and with plants growing in high P conditions still being colonised (for example, Allsopp and Stock 1994). The large degree of variation in colonisation levels, in both the glasshouse and the field (Figs. 12.2 and 12.3), may also imply that there is, generally, only a loose mutualism between the fungi and the host plant and that, therefore, benefits to the plant may occur relatively sporadically or be relatively diffuse (Fitter and Merryweather 1992) and/or that generally the costs and benefits of the relationship are close.

Fitter and Merryweather (1992) analysed data from the Ecological Flora Project, a collation of data from the entire British flora. They found that while plants with coarse roots were typically mycorrhizal, fine-rooted species were variable; indicating that factors other than ability to take up nutrients have influenced whether plants have maintained the ability to form mycorrhizas. The importance of these other factors in ensuring the VAM-plant relationship remains profitable for both partners may explain why this project found the relationship between plant P and VAM colonisation to be quite weak for rye grass — with high P having a relatively small negative effect on colonisation — compared to the coarse-rooted clover (Figs 10.2 and 10.7). It could also explain why, under high P conditions, clover maintains colonisation, even though this can potentially be detrimental to growth.

Thus, the present wide occurrence of VAM fungi can be explained as follows. The earliest land plants, with their coarse root systems, were apparently highly colonised by mycorrhizal fungi which were crucial for adequate plant nutrient uptake.

However, as the fungi could only grow through use of plant photosynthate, plants evolved the ability to reduce colonisation when nutrients were abundant. Through their presence, VAM fungi also protected plants from pathogens and perhaps aided water uptake. As plants evolved more advanced root systems, the role of VAM fungi in plant nutrition decreased. However, the ability to form mycorrhizas was generally not selected against — although some lines of mycotrophic plants did arise — as many plants derived benefits from the fungi other than constant enhanced nutrient uptake (Newsham *et al.* 1995). Consequently, the negative relationship between VAM colonisation and P levels weakened for these plants. Whether a particular plant thrived by being mycotrophic would, therefore, depend on a complex set of factors involving the comparative costs of other mechanisms for disease protection and overcoming periodic stress and the maintenance of VAM colonisation.

12.2. Broad Ecological Trends

This section considers the contributions of VAM fungi to ecosystem processes. Section 12.2.a contrasts the role of VAM fungi in the mixed farm and dairy farm systems, while section 12.2.b examines the broader trends of VAM occurrence and functioning across ecosystems. .

12.2.a. Do VAM Fungi Contribute Differently to the Functioning of Cropping and Pasture Systems?

This section contrasts the occurrence and functioning of VAM fungi in the mixed and dairy farm systems by examining the different perturbations and disturbances affecting each system and the resulting differences in vegetation and fungal community structure, plant and fungal lifecycles, nutrient availability and nutrient cycling. While it is possible that the role of VAM fungi in plant disease protection would differ between the two systems, this was not examined in this project.

(i) Perturbations and Disturbance

The frequency and severity of perturbations and disturbance differed greatly between the mixed and dairy farms (Fig. 12.11). Mixed farm paddocks alternated between 1-3 years winter cropping with summer fallows and 3-8 years annual pasture. The transition between annual pasture and annual cereal crops was dramatic with most living plants being killed by tillage or herbicides in spring and the soil being left bare for 2-8 months over summer. A number of cultivations, addition of fertilisers — and, on conventional farms, biocides — then occurred around the sowing of a crop in autumn.

In contrast, the dairy pastures were managed with the aim of sustaining year around shoot production and, therefore, milk production. This was achieved by eliminating cultivation and managing perturbations such as grazing, addition of fertilisers and summer irrigation, as well as maintenance of a mixed-species pasture which allowed active growth all year. Consequently, the perennial herbaceous community had persisted for, on average, 40 years.

(ii) Vegetation and Fungal Community Structure and Lifecycles

The annual crops on the mixed farms were fast growing, even-aged monocultures of even density and regular spacing, grown from genetically similar seed of one cultivar. The presowing cultivation and, on the conventional farms, addition of fertiliser in banded rows beneath the seed would have also contributed towards homogeneity across paddocks. In contrast, the mixed and dairy pastures had a more complex spatial structure, for instance, the perennial dairy pastures consisted of three main plant species — a legume (clover) and two grasses (rye grass and paspalum) — as well as a variety

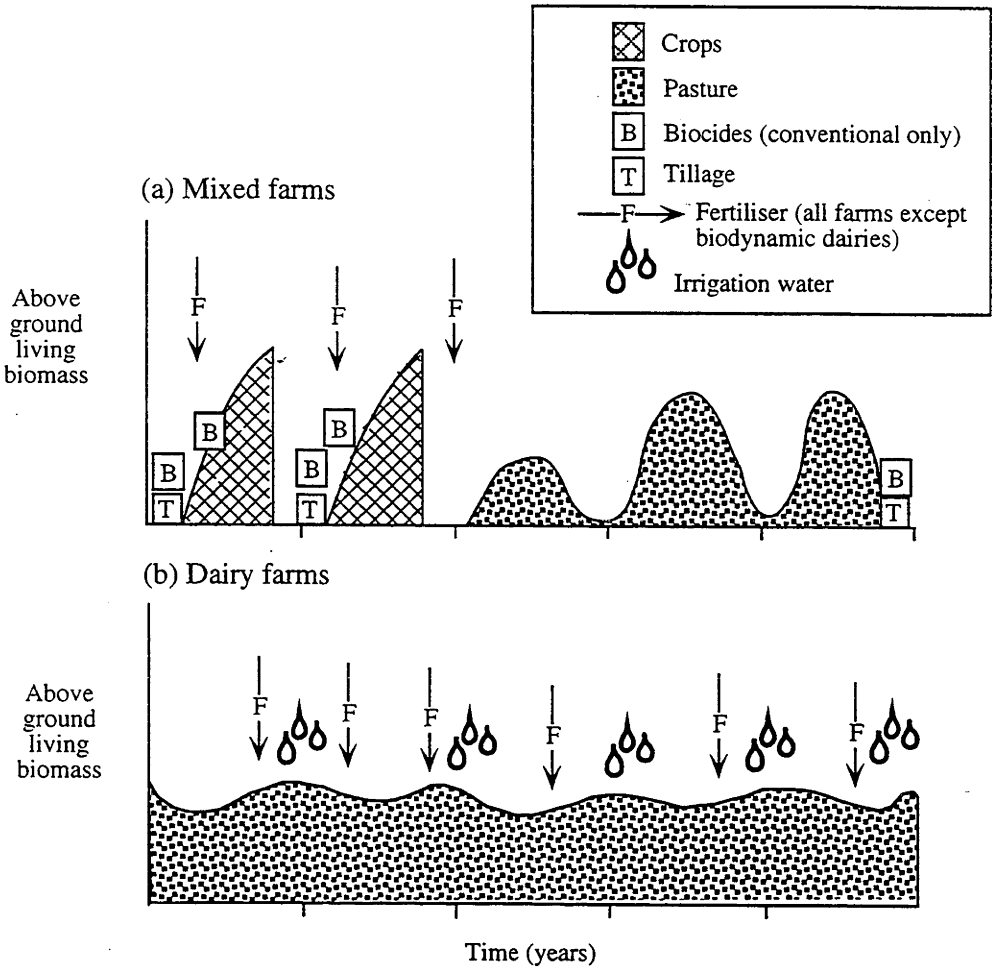


Figure 12.11. Generalised comparison over five years of the effects of the major perturbations and disturbances on an indicator of biological activity, above ground living biomass, between the mixed farms and the dairy farms.

of other grasses, sedges, rushes and weeds. The long period of time since last cultivation and the presence of irrigation check banks along contours had resulted in development of a degree of patchiness in species composition and distribution.

At the sowing of a wheat crop, due to repeated cultivation, there are generally few live shoots or roots remaining and no functional hyphal network; spores, hyphal segments and colonised root segments would act as inoculum. In contrast, in the dairy pastures, the hyphal network would quickly infect new roots; see Miller and Jastrow (1992a). Moreover, the high root density (Table 12.2), would result in new roots being rapidly colonised by external hyphae from existing active colonisation on neighbouring roots. Thus colonisation of new roots in a pasture may be quicker than in a crop and the flow of resources, either in or out of the new colony, could quickly begin.

Thus, in annual crops — and, perhaps, to a lesser degree in the annual pastures — VAM fungi must fit their lifecycle in with the seasonal cycle, initially primarily forming arbuscules and later forming vesicles and spores as the plants die and the arbuscules deteriorate (Ryan 1992; Gavito and Varela 1993; and discussion in An *et al.* 1993). This would not be so critical in the perennial pastures, where there is some plant growth all year. Overall, the contrasts between the mixed and dairy farms may have resulted in differences in VAM community structure and, indeed, these may parallel the differences present in the host plants. For instance, characteristics of the VAM community favoured on the dairy farms could include some, or all, of the following:

- a diverse range of fungal species differing in host preference and optimum season for activity;
- colonisation of new roots by hyphae from existing colonies;
- only occasional production of spores;
- low spore dormancy;
- long external hyphae to search for gaps in the dense root and hyphal matrix;
- efficient acquisition of P from organic sources;
- ability to fuse hyphae and form a hyphal network.

In contrast, the VAM community during cropping on the mixed farms may have some, or all, of the following characteristics:

- a small number of VAM species all competing for the one host and all simultaneously passing through lifecycle stages;
- copious production of spores;
- high spore dormancy broken by specific cues such as cooler temperatures, high soil moisture or presence of a living root;
- production of many short external hyphae near roots, as little competition between roots.

The alternation between crops and pastures on the mixed farms may result in selection for VAM fungi possessing a mixture of the above characteristics, or species possessing one set of the characteristics plus the ability to persist through the years when the system is favouring species with the other set.

This project did not closely examine this issue, however it is a topic worth further research. This could involve regular sampling of spores over a year and establishment of pure cultures of the VAM species present. Glasshouse trials — involving treatments varying temperature, host plants, soil water and P source — could then be conducted to compare the spore germination of each VAM species and the effects of each VAM species on host plant growth.

The perennial nature of the dairy pastures allows greater root lengths and hyphal lengths than under the crops (Table 12.2). This may result in VAM fungi making a greater contribution to the maintenance of soil structure through the formation and stabilisation of soil aggregates (Bethlenfalvay and Barea 1994; Miller and Jastrow 1990; Miller and Jastrow 1992b; Tisdall 1991). However, in terms of ecosystem maintenance, this process may be more important on the mixed farms, where better structured soil would be important for minimising erosion during the summer fallow and enabling soil to retain its structure after cultivation.

(iii) Nutrient Availability and Cycling

A summary of the nutrient concentrations in the soils and plants in each commodity production system is presented in Table 12.3. Soil extractable P and soil total N concentrations were higher on the dairy farms. On the mixed farms, both P and N had the potential to limit growth of subterranean clover in pasture, while wheat was limited by P, particularly on the alternative farms. In the dairy pastures, clover was limited by P only on farms with low soil extractable P and was unlikely to be limited by N; rye grass was strongly limited by N. As rye grass constituted 40-50% of pasture biomass (Small *et al.* 1994a), N was the nutrient most limiting for pasture growth on the dairy farms. The lower levels of soil nutrients on the mixed farms means that VAM fungi may, potentially, play a larger role in plant nutrient uptake on these farms (see Table 12.1).

Table 12.3. Summary of P and N concentrations in soil and plant shoots from the mixed farms and dairy farms (§5.4.e.ii, §6.5.a, §7.3.d.ii, §10.4.a and §11.1.d.iv). Shoot nutrient concentrations were estimated to be adequate for normal growth (+), low (-) or deficient (--).

	Mixed Farms		Dairy Farms	
	Sub-clover	Wheat	White clover	Rye grass
Soil nutrient concentrations				
Colwell extractable P ($\mu g\ g^{-1}$)	10 - 29		36 - 88	
Total N ($\mu g\ g^{-1}$)	700 - 1700		3500 - 5800	
Shoot nutrient concentrations				
Phosphorus	- - to +	- to +	- to +	+
Nitrogen	- - to +	+	+	- -

Figure 12.12 presents the major nutrient transfer paths for a crop on a mixed farm and a dairy pasture. In a crops, the major flows involve fertiliser inputs and a single large output of harvested grain; although much of the N used in a first year crop would have been biologically fixed by legumes during the previous pasture phase. On the dairy farms, fertiliser inputs and biological N-fixation from legumes are large, while the major outputs are animal products.

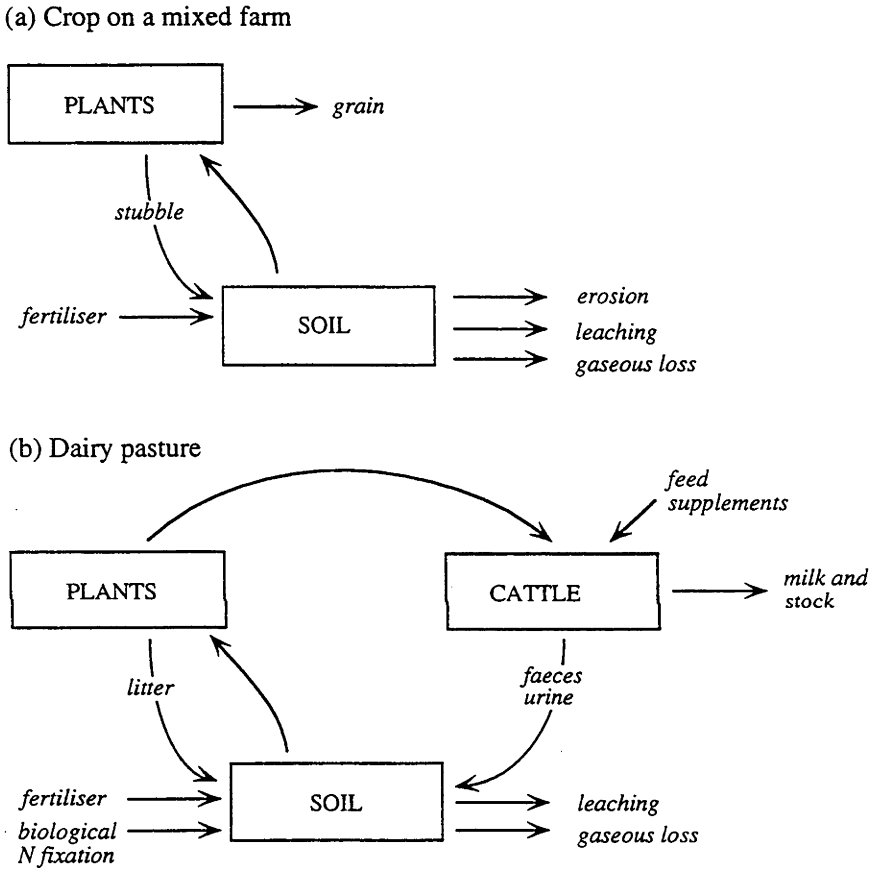


Figure 12.12. Major nutrient transfer paths in a) a crop on a mixed farm and b) a dairy pasture.

Table 12.4 contains approximate estimates of nutrient fluxes. Although many factors are not accounted for, it is clear that internal recycling of nutrients from plant material is an order of magnitude greater in the dairy pastures than in crops on the mixed farms. During cropping the return of plant material to the soil is primarily of stubble left after harvest, however, this contains few nutrients. In a dairy pasture, nutrients from plant material would continually be recycled in relatively large amounts through faeces, urine and the return of plant litter.

On the mixed farms, the overall P balance was negative on the conventional farm and, due to the addition of large quantities of P as the relatively insoluble rock phosphate and lower yield, positive on alternative mixed farm. The N balance was negative for both farms, however, the contribution of biological N fixation during the preceding pasture phase was not calculated. On the dairy farms, the P and N balances were negative on the biodynamic farm due to no fertilisers being applied, although the N balances are problematical due to the estimation of biological N fixation.

Table 12.4. Approximate inputs and outputs, internal recycling of above-ground biomass and overall balances of P and N in a first year conventional (Con.) and organic (Org.) wheat crop on mixed farms and conventional and biodynamic (BD) irrigated dairy pastures (kg ha^{-1} $year^{-1}$). The recycling of nutrients contained in plant roots and losses of nutrients through erosion, leaching and nitrification are not included.

	First Year Wheat Crop(1)			Pasture on a Dairy Farm(2)		
	Source	Con.	Org.	Source	Con.	BD
Phosphorus						
Inputs	fertiliser	16	20	fertiliser, feed supplements	30	1
Outputs	grain	20	11	milk, stock	14	9
Internal recycling	stubble	2	1	faeces and urine(3) plant litter(4)	42 10	18 5
Balance		- 4	+ 9		+ 16	- 8
Nitrogen						
Inputs	fertiliser	14	0	fertiliser, feed supplements N-fixation(4)	108 135	4 70
Outputs	grain	146	84	runoff(4), milk , stock	150	100
Internal recycling	stubble	26(5)	18	faeces and urine(3) plant litter(4)	278 550	140 225
Balance		- 132	- 84		+ 93	- 26

(1) based on the 1993 crops at Ardlethan (Derrick 1996).
(2) unless otherwise indicated, based on the NE Victoria irrigated dairy pastures (Small *et al.* 1994a).
(3) includes nutrients from pasture and feed supplements: assumes 90% of ingested nutrients are returned as faeces or urine (Haynes and Williams 1993).
(4) estimates based on data presented in Haynes and Williams (1993).
(5) assuming the stubble is not burnt.

The far greater degree of internal recycling of nutrients from above-ground biomass in the dairy pastures may involve the actions of a VAM hyphal network. VAM fungi can efficiently use organic P in the form of Na-phytate (Tarafdar and Marschner 1995). Joner and Jakobsen (1995) found that VAM colonised plants utilised a much greater amount of P released from organic matter than non-VAM plants; apparently due to VAM hyphae exploring a greater volume of soil, not to VAM enhancing mineralisation (Joner and Jakobsen 1995). Moreover, Newman and Eason (1989) suggest that VAM hyphae attached to living roots may efficiently absorb water-soluble nutrients as they leak out of dying roots and they provide evidence that direct VAM hyphal links between roots are involved in P transfer from dying to living roots.

Such by-passing of the detritus food-web may reduce the likelihood of nutrients being lost through leaching or denitrification, as well as decrease the fixing of P into unavailable forms. Even if high root densities in the top of the soil profile make the role of VAM fungi obsolete in this regard (§12.1.b.iii), the decrease in root density down the profile should make them progressively more important. However, these processes would not be important in a cropping system, as most roots die simultaneously around harvest when no plants or active VAM fungi are present.

12.2.b. Are There Predictable Relationships Between Nutrient Inputs, Concentrations of Nutrients in Soil and Plants, and Levels of VAM Colonisation Occurring Across Farm Management Strategies and Commodity Production Systems?

This section contains a discussion of the broad trends apparent from this project, and summarised in Figures 12.13 and 12.14, with reference to the few similar studies.

(i) Abundance of VAM Fungi

Figure 12.13 contains graphs of data from wheat crops sampled at 15 sites in six paddocks on the mixed farms at tillering in 1993 (Chapter 5). Figure 12.14 contains graphs using four sets of paddock averages from clover plants in pastures sampled during this project: the 19 pairs of conventional/alternative dairy farms sampled in March 1993 and March 1994 (§10.3.a.ii); the six dairy farms sampled as part of the time series in January 1994 (§10.3.b); and the eight mixed farm pastures (Chapter 6).

For both wheat and clover there was a negative relationship between VAM colonisation and both soil extractable P and shoot P (Figs. 12.13.a, b and 12.14.a, b). The outliers in the Fig. 12.14.b with high P and high VAM were all conventional and were discussed in section 10.4.b.ii. The reasons for the stronger trends shown by the dairy data were discussed in section 12.1.a.x. The negative relationship between P and VAM colonisation levels was strongly confirmed in glasshouse trials where P levels were manipulated (§7.3, §11.1 and §11.2).

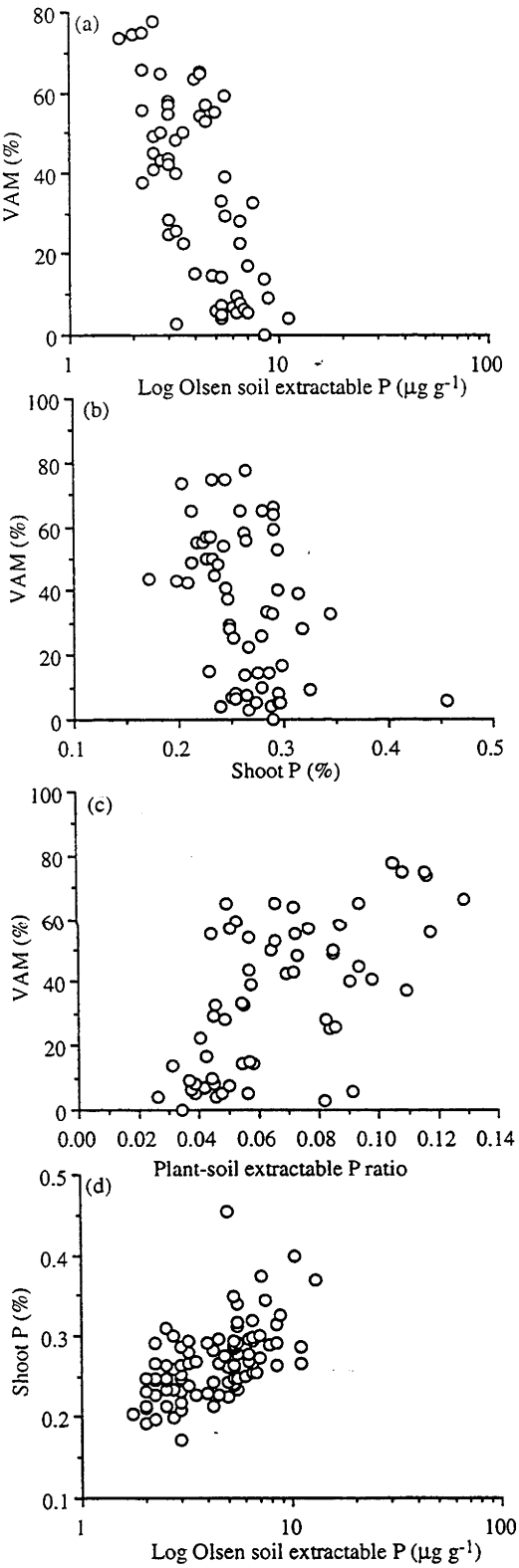


Figure 12.13. Data from 15 sites in wheat crops at tillering in 1993 on six farms. Relationship between the percentage of root length colonised by VAM fungi and a) soil extractable P, b) shoot P and c) the ratio of shoot P to soil extractable P and d) the relationship between shoot P and soil extractable P. Soil and shoot P data from Derrick (1996).

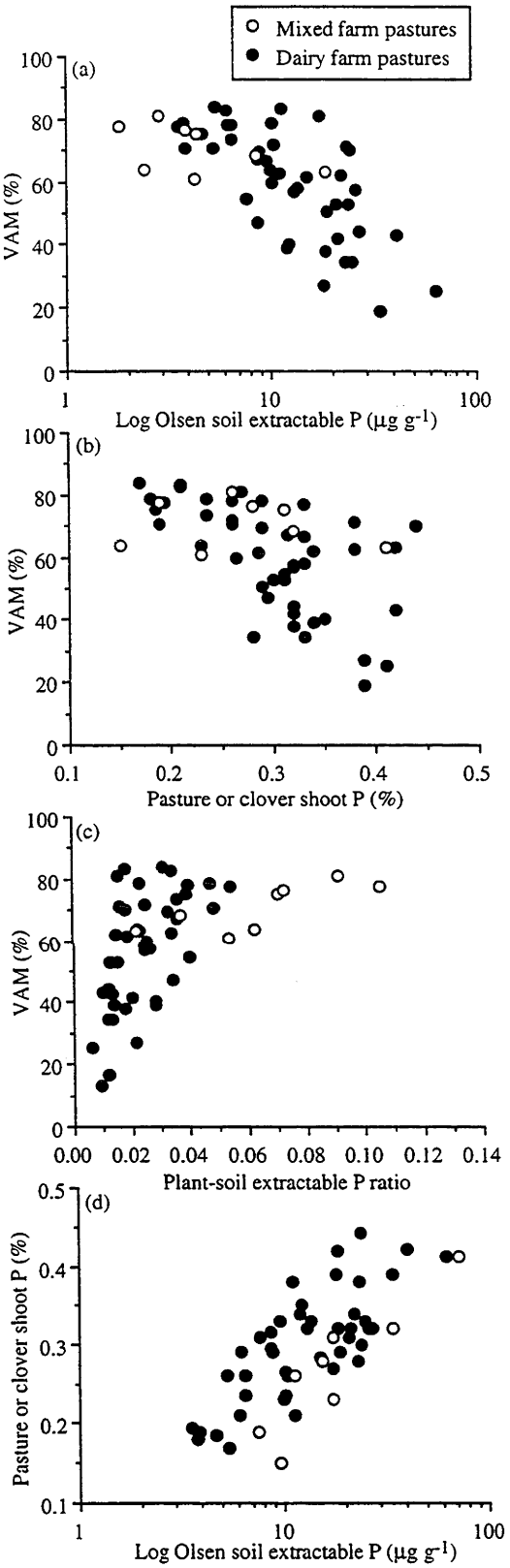


Figure 12.14. Paddock means from dairy pastures on 20 NE Victorian farms (1993), six NE Victorian farms (1994), 19 farms in other regions (1994) and eight pastures on four mixed farms (1995). Relationships between VAM (%) and a) soil extractable P, b) clover shoot P (mixed) or pasture P (dairies) and c) the plant-soil extractable P ratio and d) the relationship between shoot P and soil extractable P. Dairy P data from Small *et al.* (1994a).

A number of concordant results have been reported. For instance, Mårtensson and Carlgren (1994) measured the abundance of VAM spores in two field experiments in Sweden over 30 years. The number of spores was strongly negatively affected by additions of soluble P, while no other soil properties had an effect. Similarly, the species richness and diversity of VAM communities associated with cacao in Venezuela were negatively correlated with soil extractable P (Cuenca and Meneses 1996). Allsop and Stock (1994) examined VAM colonisation levels in three communities in South Africa, finding that average VAM colonisation levels, but not spore numbers, were negatively related to soil extractable P (Table 12.5).

Table 12.5. Soil P concentrations and VAM colonisation levels in three lowland evergreen shrublands in South Africa. Data from Allsopp and Stock (1994).

	Renosterveld	Fynbos	Strandveld
Total P ($\mu g\ g^{-1}$)	127	23-34	422
Extractable P ($\mu g\ g^{-1}$)	0.8	1.4	40
VAM spores (g^{-1})	0.9	0.38	0.94
VAM (%) in Asteraceae	70	37	32
Percentage of plants with VAM (%) >54	61	43	24

McNaughton and Oesterheld (1990) sampled VAM hyphae in soil at 35 locations across the Serengeti National Park in Tanzania, including eight different landscape types, different topographical positions within landscapes and 19 different grass species within a 12000 km^2 area. Hyphal length was negatively correlated with both soil organic matter content and total soil mineral nutrient concentration. As the concentration of all soil nutrients were highly correlated, and no manipulated experiments were conducted, it was not possible to attribute the trends in hyphal lengths to any one particular nutrient, although they are consistent with a P effect.

Brundrett *et al.* (1996) examined the VAM inoculum potential of soil from natural vegetation in northern tropical Australia. Propagule numbers varied significantly between different vegetation types and were positively correlated with a number of soil properties including water storage capacity and organic matter, but not soil extractable P which was low at all sites.

The results presented above suggest that VAM colonisation levels will generally be broadly, negatively correlated with the availability of P, lending support to the hypothesis that the VAM symbiosis originally arose as a means of enhancing plant P uptake (§12.1.c.v). Thus, within a particular ecosystem, or between a set of ecosystems, the abundance of VAM fungi can be expected to vary primarily in response to P, unless other relatively extreme environmental conditions — such as the 1994 drought — significantly limit plant or fungal growth. For sites where P concentrations

are consistently low — see for example, Brundrett *et al.* (1996) — or for sites with similar concentrations of extractable P, correlation with other environmental parameters should become evident.

(ii) The Functions of VAM Fungi

Both the wheat and clover exhibited a strong positive relationship between the plant-soil P ratio and VAM colonisation (Figs. 12.13.c and 12.14.c). This is due to the negative relationship between VAM and P and the non-linear, but positive, relationship between soil and shoot P levels (Figs. 12.13.d and 12.14.d). For the clover, for a given plant-soil P ratio, there was a well-defined lower limit for VAM colonisation and variation in VAM colonisation above this level was in response to the actual concentration of P in the pasture. Consequently, alternative farms were generally more highly colonised than conventional farms for the same shoot-soil P ratio. Wheat did not show such a well-defined lower limit (Fig. 12.13.c), presumably because the data was from individual sites in a paddock and not paddock means. Moreover, the annual nature of the crop meant that the relationships between soil and shoot P and VAM colonisation would still be changing and no equilibrium point would have been reached (§12.1.a.x).

The study by McNaughton and Oesterheld (1990), described above, also found a positive correlation between hyphal density and the plant-soil ratio of nutrients, but no significant correlation between hyphal density and the concentration of nutrients in plants (Table 12.6).

Table 12.6. Comparison of the relationships between soil P, plant P and VAM fungi between the Serengeti grasslands (McNaughton and Oesterheld 1990) and the two SE NSW agricultural systems examined in this project. Abundance of VAM fungi was measured as hyphal density in the Serengeti and as VAM (%) in this project. Wheat results use individual site data (Fig. 12.13) and pasture results use paddock averages (Fig. 12.14). Parameters were related positively (+), negatively (-) or not at all (0).

	Serengeti	Wheat	Pastures
VAM / soil P	-	-	-
VAM / plant P	0	-	-
VAM / plant-soil P ratio	+	+	+
Soil P / plant P		+	+

McNaughton and Oesterheld (1990) suggest that the positive relationship between VAM fungi and the plant-soil P ratio indicates that VAM fungi are, to some degree, buffering plant mineral contents as soil nutrient concentrations decrease. This is consistent with the strong growth response of clover to VAM colonisation in the low P mixed farm soils and the smaller response in the relatively high P dairy soils (Table 12.1). However, the strong positive relationship between soil P and plant P in this

project (Figs. 12.13.d and 12.14.d) indicates that VAM fungi did not even come close to totally buffering the effects of variations in soil extractable P. This may result, in part, from the agricultural systems containing plants bred to respond to additions of P and N fertiliser and the systems being manipulated to reduce the influence of other limiting factors, such as disease, water, interspecific competition and grazing.

Thus at a broad ecosystem level, VAM fungi will be most abundant when nutrient availability is low and may, therefore, act to stabilise nutrient fluxes, thereby helping to maintain plant nutrient concentrations within relatively narrow levels and reducing the potential for nutrient stress in plants and herbivores. Such a relationship could be summarised as shown in Figure 12.15.

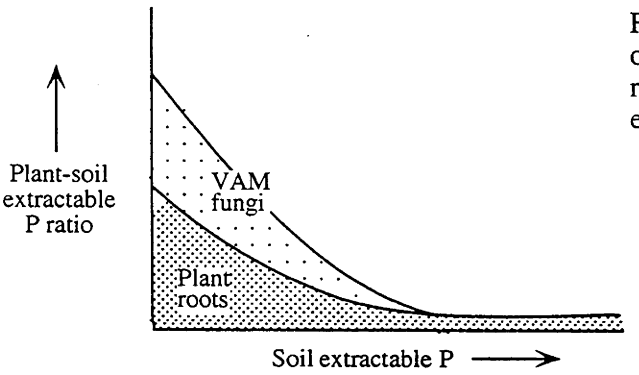


Figure 12.15. The relative contributions of VAM fungi and plant roots to the relationship between the plant-soil extractable P ratio and soil extractable P.

(iii) Effect of Scale on the Functioning of the VAM Fungi-Host Plant Relationship

This project has shown that VAM fungi interact with their surrounding biotic and abiotic environment at a number of scales. While these interactions are extremely complex and involve many feedback mechanisms — see diagrams in Miller and Jastrow (1994) and Allen *et al.* (1992) — they may be summarised as shown in Table 12.7.

At the ecosystem level, environmental characteristics may also influence which type of mycorrhizal association — VA mycorrhizas, ectomycorrhizas or ericoid mycorrhizas — will predominate (Read 1991). Identification of parameters that correlate to the broad scale occurrence of VAM fungi, and the relating of these to the role of VAM fungi in ecosystem functioning through an understanding of the functioning of VAM fungi at finer scales, is essential if the role of VAM fungi in global processes, such as the carbon cycle and climate change, is to be estimated.

Table 12.7. Major factors affecting VAM fungi and the effects of VAM fungi on their surrounding environment and host plants at various scales.

Scale of structural organisation	Factors affecting VAM fungi	Effects of VAM fungi
VAM species	Environmental conditions, disturbance, resources, host availability	Fungal interspecific competition
Plant species	Root architecture, level of mycotrophy, annual or perennial lifecycle	Plant distribution and abundance
VAM association: individual VAM fungus	Factors affecting germination and growth of spores and propagules (especially P and water)	Soil structure, effects on soil fauna
VAM association: individual plant	Plant P and N concentration, factors affecting plant photosynthate production (water, light, grazing)	Plant nutrition (especially P), biomass, root-shoot ratio, other factors associated with growth and reproduction, disease resistance
Community	Frequency of mycotrophic plants, grazing, root density, intraspecific and interspecific competition	Outcomes of competition, effects on insects and grazers, linking of plants through hyphal network
Ecosystem	P availability, water availability, disturbance levels and frequency of disturbance	Buffer plant nutrient levels, reduce nutrient stress in plants and herbivores, tighten nutrient cycles, primary production levels

12.3. Comparisons Between Farms Under Conventional and Alternative Management

12.3.a. Do Plants on Alternative Farms have Higher Levels of VAM Colonisation?

Levels of colonisation by VAM fungi, in both crops and pastures, were consistently higher on alternative farms than on conventional neighbours. This was primarily due to the use of relatively large volumes of soluble P fertilisers on the conventional farms. The strong negative correlation between plant P and VAM colonisation in the field — along with the strong negative effect of P additions on colonisation in the glasshouse trials — implied that other factors associated with conventional agriculture, such as use of herbicides and pesticides, were not consistently significantly reducing VAM colonisation. They may, however, have affected the functioning of the fungi (Dehn *et al.* 1990; Kough *et al.* 1987; §12.1.a). Similarly, the strong relationship between VAM colonisation and P implies that practices associated with alternative agriculture, including the application of substances specifically designed to enhance microbial activity — such as BD 500 — did not significantly increase VAM colonisation.

Overseas studies have also consistently found VAM colonisation levels to be higher on alternative farms than conventional neighbours (Bokhorst 1989; Gruhn 1996; Lengnick and King 1986; Miller and Jackson 1996; Sattelmacher *et al.* 1991; Werner 1997; Werner *et al.* 1990). In some instances, lower VAM colonisation on the paired conventional farms was associated with higher P levels (Sattelmacher *et al.* 1991), however this was not always the case (Werner 1997). Other practices of conventional agriculture were also suggested as reducing VAM fungi, including fungicide applications (Werner *et al.* 1990), various crop rotations (Sattelmacher *et al.* 1991), pesticide applications (Miller and Jackson 1996; Werner 1997) and the absence of vegetation around orchard trees (Werner 1997). These conclusions were generally inferred — not experimentally tested — and although such factors may reduce VAM colonisation (for example, Menge 1982 and Ocampo 1980), results are often variable; particularly the effects of agricultural chemicals (§12.1.a.ix).

Thus, while many of the factors associated with conventional agriculture have the potential to reduce VAM colonisation (Table 1.1), the addition of fertilisers containing soluble P is probably the most common cause of VAM colonisation levels being lower on conventional farms than on alternative farms; particularly in Australia where soil extractable P often limits plant growth (§2.2) and most conventional farmers apply soluble P fertilisers.

12.3.b. Are Plants on Alternative Farms more Reliant on VAM Fungi for Nutrient Uptake?

On the mixed farms, growth of the major crop, wheat, exhibited no net benefit from VAM colonisation, even on low-P soils where P was strongly limiting wheat growth (§7.3.d.iii). In an examination of crops in the NE Australian wheatbelt with varying levels of VAM colonisation due to being planted after short or long fallows, Thompson (1987) also found wheat to be relatively non-dependent. However, other crops — including sunflowers (*Helianthus annuus* L.), sorghum (*Sorghum bicolor* (L.) Moench), chickpeas (*Cicer arietinum* L.), sudan grass (*Sorghum sudanense* (Piper) Stapf) and maize (*Zea mays* L.) — had significantly lower growth when VAM colonisation was reduced following a long fallow.

The winter-cropping mixed farms in the lower-rainfall southern wheatbelt, sampled in this project, do not usually grow any of these crops. Other than cereals, the only crops routinely grown are the non-VAM canola (*Brassica napus* L.) and lupins (*Lupinus* spp.). Indian mustard (*Brassica juncea* L.), also a non-VAM plant, is the only crop likely to be widely adopted on these farms in the foreseeable future (J. Angus, pers. comm.). Thus, in terms of maximising crop yield, conventional and alternative farmers in the southern wheatbelt do not need to consider VAM fungi. Even if inoculum levels are markedly reduced, as occurred in the 1994 drought (Chapter 8), this is unlikely to have an adverse effect on yields of subsequent crops.

In contrast, subterranean clover, the main legume component in the pastures on the mixed farms, is dependent on VAM fungi. The relatively low concentration of soil extractable P — including on conventional farms (Table 6.4) — ensures that when a paddock is returned to pasture, colonisation levels will quickly reach high levels on all farms. Thus, both conventional and alternative farms will receive any benefits that VAM fungi provide for plant nutrition.

In the glasshouse trial conducted using biodynamic dairy farm soil (§11.2), growth of white clover, rye grass and paspalum was not greatly increased by VAM colonisation. However, unexpectedly, the soil contained 43 $\mu g\ g^{-1}$ of Olsen extractable P; a concentration similar to that on the conventional dairy farms and around twice as high as the average on the biodynamic farms (§11.2.b.i). When P was added, in a simulation of conventional farm fertiliser practices, the effect of VAM fungi on plant growth tended to decrease (Fig. 12.7). Thus, on the dairy farms, VAM fungi may be most significant in enhancing the nutrition and competitive ability of white clover, especially in the lower-P biodynamic soils. However, the conventional farms do have quite high levels of fungal colonisation and fungal hyphae (Table 12.2) and thus, as in the mixed farm pastures, all farms would receive any benefits, nutritional or otherwise, that are associated with VAM colonisation.

Thus, plants on alternative farms will, generally, be more reliant on VAM fungi for plant P uptake than plants on conventional farms where soluble P fertilisers are applied and, consequently, soil extractable P is higher. Alternative farms, by simply eliminating soluble P fertilisers, will usually have close to the maximum level of VAM colonisation and, therefore, farmers do not need to plan farm management to manipulate levels of VAM fungi. However, this will not be sufficient to increase plant growth to the levels achieved on conventional farms using soluble P fertiliser (Fig. 5.6). On the conventional farms, VAM fungi would need to be significantly influencing processes other than plant nutrient uptake, such as decreasing pathogen levels or closing nutrient cycles, before the yield decline associated with reducing additions of soluble P fertilisers to increase VAM colonisation would be acceptable. However, these other processes may eventually be considered of greater importance as it becomes necessary to reduce the environmental impact and increase the long-term sustainability of agricultural systems (§12.3.d).

12.3.c. Do the Soil Processes Associated with Plant Nutrient Uptake on Alternative Farms Differ Substantially From Those on Conventional Farms?

It has been suggested that organic and conventional agriculture belong to two different paradigms and consequently the ability of conventional agricultural science to explain the processes operating on alternative farms may be inadequate (Wynen 1996). An enhanced role for soil organisms in ecosystem processes on alternative farms is often stated to be part of this difference (§2.3.c). This section examines the validity of such claims in view of the results from this project and other studies which compared conventional and alternative farms.

(i) Factors contributing to Plant Growth on the Farms in this Project

Using stepwise regressions (§3.9), this project found that the functioning of alternative farms was not consistently significantly affected by processes, biological or otherwise, which differed from conventional farms (§5.4.f and §10.4.e). There was only one example of the addition of farm management strategy greatly increasing the amount of variation accounted for by a regression model. This was the relationship between VAM (%) and soil extractable P and soil total N in the 1993 wheat crops (Table 5.13). It appeared that VAM colonisation levels were either higher on the alternative farms or lower on the conventional farms than would be expected given the soil extractable P and soil total N concentrations on each set of farms. Most likely, this reflected wheat plants on the conventional farm at tillering obtaining most of their P from the banded fertiliser under the crop rows, while the measure of soil extractable P was an average from the rows and inter-rows (§5.4.b.ii). Thus VAM colonisation on the conventional farms was lower than would be expected from the soil extractable P measure. This was the only instance where such an anomaly occurred.

The glasshouse trial in Chapter 11 compared the response of plants and VAM fungi to nutrient additions in soil from three conventional/biodynamic farm pairs, finding no indication of the conventional and biodynamic soils responding differently to nutrient additions, even though the farms had differed in their fertiliser applications for many years (§11.1.d.iv).

Biodynamic farmers consider that their farms operate through processes which differ from those on both conventional and organic farms (various farmers, pers. comm.). However, again, the results from the 1993 wheat crops and the dairy pastures gave no indication that this was the case. Thus, in general, all farms apparently operated through similar processes, with any differences in the level of activity of any particular process simply due to different types and amounts of inputs.

It is also claimed that a number of years are needed before a conventional system adapts to alternative management and, consequently, that yield reductions may occur in the initial years until soil biological activity increases and the system reaches a new equilibrium; a 'conversion period' (Janke *et al.* 1991; Wynen 1992; Wynen 1996). While the conversion crop of wheat at Ardlethan in 1993 had a lower yield than its conventional neighbour, due to not applying soluble P fertilisers, there was no evidence that this was exacerbated due to the absence of processes which were present in the crop on the longer established neighbouring organic farm (§5.4.f).

It is possible that the broad measures used in the stepwise regressions and glasshouse trial may have resulted in subtle differences in the functioning of the farms being masked. Such differences, although relatively minor in one season, may eventually influence the long term sustainability of the farms (see discussion on soil structure in §12.3.d).

(ii) Variations in VAM Species and Function

It is well established that the population structure of VAM fungal communities significantly changes in response to agricultural practices such as tillage (Douds *et al.* 1995) and rotation history (Hendrix *et al.* 1995; Johnson *et al.* 1992). It has also been suggested that VAM species composition may vary in response to P fertiliser addition and that VAM fungi in unfertilised soil may become more effective at aiding plant P uptake than those in fertilised soil (Johnson 1993; Scott *et al.* 1996). Johnson (1993) found that eight years of P and N fertilisation altered the species composition of VAM fungal communities, as assessed by spore numbers. In a glasshouse trial with addition of N and P fertiliser, the number of inflorescences on big bluestem grass (*Andropogon gerardi* Vitm.) after three months was approximately 3.5 in a non-VAM control, 10.5 in plants inoculated with soil from the unfertilised plots in the field and 8 in plants inoculated with soil from fertilised plots; all significant differences. Thus inoculation with VAM species from fertilised soil decreased inflorescence numbers by 25%

compared to inoculation with species from fertilised soil. Johnson (1993) concluded that fertilisation favoured VAM species less capable of improving plant growth. In contrast, Cooper (1978) found VAM fungi from high P soils were better at colonising and stimulating the growth of clover over a range of soil P concentrations.

In this project, the examination of VAM spores from the dairy farms indicated that, after 15 years of differing P fertiliser applications, the VAM species composition did not vary significantly between conventional and biodynamic farms. The methods of spore extraction and identification used in this project were, however, relatively rudimentary (§10.4.c). It is possible, therefore, that more rigorous investigation would reveal differences in species composition. It is also possible that the VAM fungi present on the conventional and biodynamic farms differed in their functioning, as isolates of the same species may behave differently (Graham *et al.* 1982). However, in glasshouse trials using both mixed farm and dairy farm soils (§7.3 and §11.3), inoculation with different VAM species did not result in different effects on plant growth.

Figure 11.8 indicated that VAM fungi on a conventional dairy farm had not evolved to be more tolerant of P, in terms of colonisation levels, than the fungi from a neighbouring biodynamic farm. This appears to contrast with the findings of Jasper *et al.* (1979) where the relationship between VAM colonisation and shoot P differed between rye grass grown in soil from virgin land and rye grass grown in soil from adjacent cultivated land which had received regular P applications for 30 years. As VAM colonisation was consistently higher at any given shoot P concentration for plants in the cultivated soil, it was concluded that the VAM fungi in the virgin soil were more sensitive to P (Jasper *et al.* 1979; Fig 12.16).

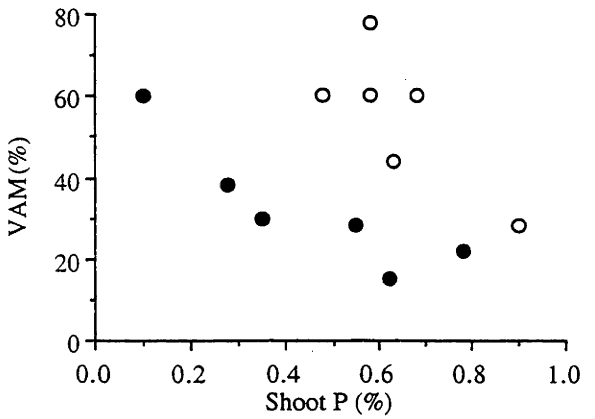


Figure 12.16. Percentage of rye grass root length colonised by VAM fungi when grown under six levels of P addition in virgin soil and in cultivated soil from a adjacent paddock which had received regular soluble P fertiliser applications for 30 years. Adapted from Jasper *et al.* (1979).

However, as Jasper *et al.* (1979) found only spores of *Acaulospora laevis* in the virgin soil and only spores of *Glomus monosporus* in the cultivated soil, the different response to shoot P could well have resulted from the differing colonisation characteristics of the two species (see Fig. 11.14).

The effects of P fertilisers on VAM species composition and the influence of differing species or isolates of VAM fungi on plant P uptake are areas meriting further research. Development of good identification procedures for VAM fungi would aid such research. The possibility that the effects on plant growth of changes in VAM species composition may be less under field conditions than in the glasshouse, due to different conditions for plant growth or different VAM species dominating colonisation, should be considered (§12.1.b.ii). The dairy soils, where P inputs have differed for >17 years, provide an opportunity for further experimentation.

(iii) Rhizobium Bacteria

This project briefly examined levels of *Rhizobium* nodulation in clover. Clover roots on conventional farms had more frequent nodules (Table 10.6), apparently due to higher P concentrations (Fig. 10.8 and Table 11.6). The implications of this for plant growth were not assessed. However, in soils where P limits plant growth, it is possible that alternative farms may have lower rates of N-fixation than conventional farms.

(iv) Case Study: Ardlethan Fertiliser Trial

The complexity of the soil ecosystem makes it difficult to assess the contribution of individual components to the functioning of the entire system. Even if a difference in the soil ecosystem between conventional and alternative farms is identified, it may not be possible to draw conclusions about the effect of this on the functioning of each type of farm. Thus, it may be useful to measure the functioning of the entire system.

This was attempted by Dann *et al.* (1996) who examined wheat growth on the conventional (I) and organic farms at Ardlethan. The organic farm had ceased applying soluble P fertilisers 30 years previously and had subsequently been applying poorly soluble rock phosphate. It was speculated that the organic soil ecosystem may have adjusted to allow plants to access P from the rock phosphate, while on the conventional farm, biological mechanisms allowing plants to access P from rock phosphate would be absent, or less efficient than on the organic farm.

On each farm, for two consecutive seasons, wheat grown under four rates of superphosphate and rock phosphate were compared with an unfertilised control; P was the major limiting nutrient for crop growth and yield. In 1991, a relatively dry year, there was little difference in yield between the trials on each farm. In 1992, a wetter year, yields were significantly higher on the conventional farm (Fig. 12.17) in all

treatments. In both years, the addition of superphosphate increased crop growth and yield on both farms, while the addition of rock phosphate had no significant effect.

Rock phosphates have been found to enhance yield under conditions of relatively low soil pH (pH < 6, in water) and high rainfall (> 800 mm yr^{-1}) evenly distributed throughout the year. Even under these conditions, however, they may be more suited to permanent pastures than to annual pastures or crops (Bolan *et al.* 1990).

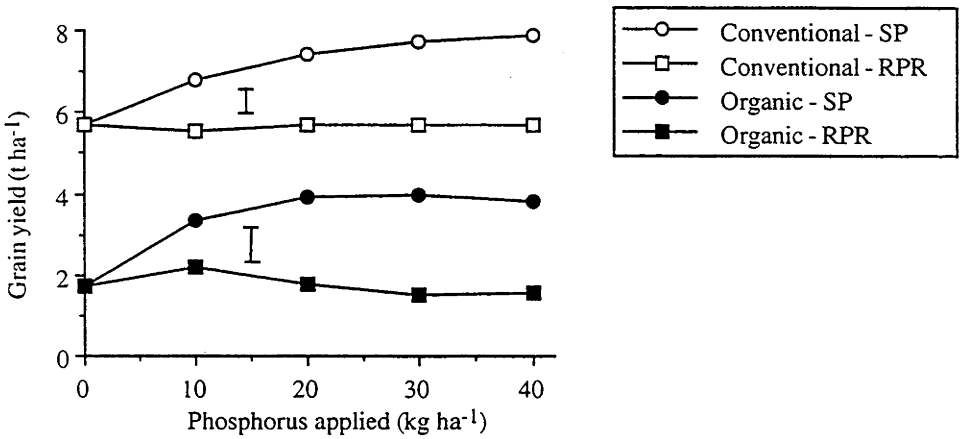


Figure 12.17. Grain yield in fertiliser trials on the conventional (I) and organic farms at Ardlethan in 1992. Treatments were a control and four levels of fertiliser added as either reactive phosphate rock (RPR) or superphosphate (SP). The LSD at p=0.05 is given separately for each trial. Soil extractable P concentrations (Bray no 1) were 15.0 μg g^{-1} on the conventional site and 4.8 μg g^{-1} on the organic site. Adapted from Dann *et al.* (1996).

Even with addition of soluble P fertiliser, yields on the organic farm did not approach those on the conventional farm. This could have been due to the lower background level of soil extractable P on the organic farm — wheat may take up the majority of its P from sources in the soil not added in the current season (McLaughlin *et al.* 1988) — or other factors limiting production on the organic farm, such as disease.

Thus, after 30 years of organic management, there was no evidence that the soil ecosystem on the organic farm was able to make the poorly soluble P in rock phosphate available to the crop, let alone to a greater degree than the conventional soil ecosystem. This was the case even though the wheat on the organic trial site was significantly more colonised by VAM fungi than the wheat on the conventional trial site (Fig. 5.17). However, the ability of VAM fungi to access relatively insoluble sources of P, such as rock phosphate, is controversial (Bolan 1991). Moreover, the results from the glasshouse trials in Chapter 7, suggest it is unlikely that VAM fungi enhance uptake of P by wheat in these soils (§7.3).

These results do not preclude the possibility of other mechanisms having being present on the organic farm — but absent or reduced on the conventional farm — which allowed other pools of soil P to be accessed. For instance, in a field trial on a red earth near Wagga Wagga, NSW, Whitelaw *et al.* (1997) found that inoculation with *Penicillium radicum* increased wheat yield by 14%; perhaps due to soil P solubilisation. Inoculation with *Aspergillus fumigatus* fungi and *Enterobacter* sp. and *Bacillus subtilis* bacteria have been found to enhance P uptake by VAM fungi (Tarafdar and Marschner 1995; Toro *et al.* 1997).

(v) The Importance of the Soil Biota for the Functioning of Conventional and Alternative Farms

In a review of 18 studies comparing conventional and alternative systems (Appendix 6), Ryan (1997) concluded that total soil microbial activity may vary between conventional and alternative farms, with alternative farms having higher levels under some circumstances (see also Wander *et al.* 1995). When alternative farms did have higher levels of microbial activity it was generally in response to specific farm management practices — particularly organic matter additions (Sivapalan *et al.* 1993; Werner and Dindal 1990) — not necessarily the exclusive domain of alternative farming. Due to the extensive nature of much of Australian agriculture (§2.3), large inputs of organic matter in the form of compost — commonly applied on alternative farms overseas — or in other forms, generally do not occur. Consequently, organic matter inputs generally do not vary greatly between conventional and alternative farms in Australia, or may be greater on conventional farms, due to their higher production levels (§12.3.d.ii). Consequently, total soil microbial activity is unlikely to differ between conventional and alternative broadacre farms in Australia. For instance, on the NE Victorian dairy farms sampled during this study, average soil microbial biomass was identical on the conventional and biodynamic farms (Small *et al.* 1994a).

Even if no differences are apparent in broad measures of soil ecosystem activity, the abundance of individual species or functional groups may vary in response to management strategy; although again, organic matter inputs are likely to play an important role. For instance, in a Californian apple orchard, Werner (1997) reported earthworm abundance and biomass had increased under organic management and speculated that this reflected greater compost inputs and growth of weeds under orchard trees on the organic sites. However, on the NE Victorian dairy farms, Small *et al.* (1994a) found a significantly higher biomass of earthworms on the conventional farms, 87 g m^{-2}, compared with 59 g m^{-2} on the biodynamic farms. This was attributed to lower P levels on the biodynamic farms leading to lower production levels and lower faecal inputs from cattle; longer irrigation intervals over summer on the biodynamic farms may also have negatively affected worm abundance.

Thus, the higher levels of soluble P fertiliser addition and subsequent lowering of VAM colonisation levels on conventional farms may be the greatest consistent difference between the soil ecosystems of conventional and alternative farms in Australia (Ryan 1997). The effect of this on ecosystem functioning is likely to vary between agricultural systems and host plants (§12.3.b).

Overall, there is no evidence that alternative agricultural systems are functioning through fundamentally different mechanisms to conventional systems or that the processes on alternative farms are not quantifiable using current research methods. That is, the nature of the biological interactions and pathways governing processes such as plant nutrient uptake, appear the same on conventional and alternative farms, but vary along a continuum — in a predictable manner — in response to quantifiable factors such as concentrations of soil available nutrients. Eliminating an input, such as soluble fertilisers or pesticides, is likely to change the soil ecosystem and *may* increase the role of soil organisms in plant nutrient uptake, however this is unlikely to totally compensate for any decreases in yield.

12.3.d. Is There a Role for VAM Fungi in Increasing the Sustainability of Agricultural Systems?

(i) The Importance of Phosphorus

Rock phosphate, the basic constituent of all P fertilisers, is a non-renewable resource. At current rates of usage the world reserves should last for 700 years, however, the rate of usage is likely to increase markedly in the future and environmental constraints on mining may become increasingly important (Cook 1982). There are also environmental concerns with the use of P fertilisers in Australia. Soil erosion has deposited P into streams and under certain conditions this may be released, increasing the P concentration in water and leading to growth of algae normally limited by P (McLennan 1996). This may result in a decreased diversity of aquatic life, deoxygenation of deeper water and water becoming toxic to animals (McLennan 1996). Thus, sustainable agricultural systems must aim to minimise both inputs and outputs of P.

As agricultural systems continually lose P through export of produce, it is necessary that inputs of P do occur. For instance, on the biodynamic dairy farms, the lack of P fertiliser applications has resulted in a negative P balance and a decline in total soil P (Tables 12.4 and 12.8). One way to reduce the losses, through fixation or leaching of plant available P applied in fertiliser, is to apply P as a poorly soluble compound which will have a lower rate of reaction with soil constituents. The alternative mixed farms applied the relatively insoluble rock phosphate and while this contributed towards maintaining a positive P balance on these farms, it did not influence crop yields in the year of application (Dann *et al.* 1996; Fig. 12.17).

Table 12.8. Average total P ($\mu g\ g^{-1}$) in the soil profiles from 10 pairs of conventional/biodynamic dairy farms in NE Victoria (Small *et al.* 1994a). Only at the 0-100 mm soil depth did the conventional and biodynamic farms significantly differ.

Depth (mm)	Conventional	Biodynamic
0-100	640	500
100-200	310	280
200-600	240	240

On Australian conventional farms, P applied in soluble forms in fertiliser has been accumulating in soils as unavailable, insoluble compounds. However, the ability of VAM fungi to access relatively insoluble forms of P is controversial (Bolan 1991) and the results from the fertiliser trials discussed above (§12.3.c.iv) did not indicate that VAM fungi accessed P from rock phosphate. Research into other methods for accessing this stored P — such as P-solubilising bacteria (Whitelaw *et al.* 1997) or use of crops, such as lupins, which release fixed P through secreting citrate (Stevens 1997) — may be more profitable. Thus the contributions of VAM fungi to reducing losses of P through fixation or leaching and the closing of nutrient cycles (§12.2.a.ii) may be the most important role for VAM fungi in regulating P in sustainable agricultural systems.

(ii) Soil Structure

The higher levels of VAM fungi on alternative farms may have long term benefits for the sustainability of the farms, as the external hyphae of VAM fungi have been implicated in the enhancement and maintenance of soil aggregation (Bethlenfalvay and Barea 1994; Tisdall 1991; Tisdall and Oades 1979; Tisdall and Oades 1982). Fungal hyphae can form and stabilise soil aggregates by physical entanglement, secretion of organic and amorphous materials that cement particles together and by sorption of clays to hyphae (Gupta and Germida 1988; Tisdall 1991).

A study by Gatehouse (1995) of wheat crops at Ardlethan in 1995 found greater water-stability of aggregates and a higher density of fungal hyphae on the organic farm than on the conventional (III) neighbour. There was a strong, positive relationship between water-stable macroaggregates (> 0.25 mm) and hyphal density. The organic crop also had better soil structure, including better water stable aggregation, higher porosity and faster infiltration rates. A glasshouse trial found the application of soluble P fertiliser significantly reduced hyphal length and the occurrence of water stable macroaggregates. While no direct causal relationship between VAM fungi and soil structure was established, it is likely that the VAM fungi contributed towards the better soil structure on the organic farm.

In general, the higher levels of VAM fungi in alternative crops may, to some degree, compensate for the greater number of cultivations that may be necessary for

weed control, as minimum tillage techniques involving use of herbicides cannot be adopted. The high level of VAM colonisation during the pasture phase on both conventional and alternative mixed farms probably significantly contributes to the positive effects of pasture on soil structure (Murphy and Harte 1992).

(iii) Fuelling the Soil Ecosystem

Soil organic matter levels are decreasing on many Australian farms, with associated deleterious effects on soil structure and nutrient availability (Davidson 1986; Grace *et al.* 1995). Both VAM hyphae and roots are a diversion of organic matter away from above-ground biomass, which is harvested and removed from the system, to below-ground biomass. While this movement of plant photosynthate into the soil could potentially result in a short-term yield decline, it may contribute towards the long-term maintenance of agricultural systems, through beneficial effects on soil structure and soil organisms. For instance, VAM fungi are an important food source for many soil organisms (Brundrett 1991).

The alternative farms in both the mixed and dairy systems had greater densities of roots and VAM hyphae than their conventional neighbours (Table 12.2). However, due to higher production levels, the organic matter inputs to the soil in the form of crop stubble (assuming it is not burnt) and plant litter will be far greater on the conventional farms (Table 12.4). This is reflected in the soil organic carbon levels. On the mixed farms at Ardlethan, after 30 years of differing management, soil organic carbon levels were 1.06% on the conventional farm and 1.17% on the organic farm; a non-significant difference (Derrick 1996). On the NE Victorian dairy farms, the conventional farms averaged 2.57% and the biodynamic 2.62%; again, a non-significant difference (Small *et al.* 1994a). The higher levels on the dairy farms emphasis the importance of pasture in maintaining soil organic carbon (see also Grace *et al.* 1995).

Thus, in the absence of large inputs of organic matter in forms such as compost, neither conventional or alternative management would appear superior for maintaining soil organic carbon levels or providing energy to the soil ecosystem.

(iv) Commercial Use of VAM Fungi

Plants in most agricultural systems will be quite highly colonised by indigenous VAM fungi, particularly if large amounts of fertilisers containing soluble P are not used. In this project, VAM colonisation enhanced plant growth and, similarly, in NE Australia, VAM colonisation is beneficial to many crops (Thompson 1987; Thompson 1994a). However, this may not always be the case, as many agricultural systems will be relatively fertile due to decades of fertilisation, may contain plant species bred for disease resistance — which may also have lower VAM colonisation levels (Toth *et al.*

1990) — and may be based on crops, such as cereals, with finely branched root systems. The effects of VAM colonisation in such systems may not always be positive.

For instance, in Minnesota, USA, Johnson *et al.* (1992) found that the dominant VAM species may be less beneficial, or indeed detrimental, to the crop in which they proliferate, in comparison to subsequent crops of different species. They suggest this may be part of the yield decline associated with continuous cropping. Moreover, in Kentucky, USA, the VAM fungus *Glomus macrocarpum* is responsible for the disease Tobacco Stunt which may reduce growth and yield of tobacco (*Nicotiana tabacum* L.) (Hendrix 1985; Jones and Hendrix 1987). Extractable P (Bray no.1) was 78 and 302 kg ha^{-1} in fertilised and unfertilised soil respectively; both considered high levels of P (Jones and Hendrix 1987). These studies indicate that the response to VAM fungi in agricultural systems is variable and must be assessed for each location and host plant.

Large-scale introductions of favourable VAM species in broad scale agriculture are limited by the difficulties with culturing an obligate symbiont to produce large amounts of inoculum. Even if this were to be overcome, problems would still be encountered with the ability of the introductions to compete with the indigenous fungi (Sen *et al.* 1989) and the ability of VAM fungi to increase plant nutrient uptake in high fertility agricultural systems (see above). Although other effects of VAM fungi on agricultural systems, particularly disease control and contributions to soil structure, may ultimately be more important than enhancing plant nutrient uptake.

Currently, VAM fungi in agricultural systems can only be manipulated through farm management practices and, therefore, it is necessary to know the effects of these practices on VAM populations. For instance, the promotion of tobacco yield by rotation with fescue (*Festuca arundinacea* Schreb) has been attributed to the effects of fescue on propagules of the pathogenic VAM fungus G. *macrocarpum*. (Guo *et al.* 1992). Fescue is host to the endophytic fungus *Acremonium coenophialum* which produces metabolites toxic to VAM fungi (Guo *et al.* 1992). It is possible that VAM populations could be manipulated by rotation with fescue varieties which are either susceptible or non-susceptible to the endophyte (Hendrix *et al.* 1995). Inclusion of non-VAM brassicas in rotations could also be used to reduce the VAM inoculum potential of soil (Schreiner and Koide 1993) and inclusion of highly colonised legumes as crops or pasture components could be used to increase VAM inoculum potential. In the cropping systems in this project, the effects on VAM populations of non-VAM hosts — especially, canola (*Brassica napus* or *B. campestris*) and lupins (*Lupinus* spp.) — and continuous cropping without pasture phases, are yet to be evaluated.

(v) Are Alternative Agricultural Systems More Sustainable?

Evidence is emerging that modern agricultural systems are contributing towards a number of major environmental problems. In Australia, there is concern over the role of agriculture in soil erosion, soil acidity, dryland salinity, salinisation of irrigated land, soil structural decline, pesticide residues in food, pollution of water courses and degradation or destruction of native ecosystems (Conacher and Conacher 1995; Dumsday *et al.* 1990; Hamblin 1991). For instance, in the state of Victoria, irrigation related salinity has been estimated to affect 28% of irrigated land, while 40% of farmland used for cropping or mixed operations and 89% of land under irrigated pasture is estimated to have experienced severe soil structural decline (Office of the Commissioner for the Environment 1992). The increasing recognition of the negative effects of conventional agriculture has led to interest in alternative agricultural systems from the farming community, consumers, researchers and government bodies (Dumaresq *et al.* 1997).

This project has shown that alternative agricultural systems will generally have greater levels of VAM colonisation than conventional systems. Indeed, in terms of the soil ecosystem, this may be the greatest contrast with conventional farms (§12.3.c.iv) and may enhance the sustainability of alternative farms in a number of ways, including the benefits of VAM hyphae for soil structure and the closing of nutrient cycles. However, in terms of yield, VAM fungi are unlikely to compensate for the non-use of soluble P fertilisers, unless they contribute to the plant overcoming a deficiency of another nutrient or a disease (§12.3.b).

The relative sustainability of alternative and conventional agricultural systems is a complicated and controversial topic. Table 12.9 summarises, for the two farming systems and three management strategies examined in this project, some of the many factors that may influence their long-term sustainability.

A major impediment to sustainable production on the mixed farms is the loss of soil through erosion and soil structural decline. Minimum tillage techniques have been developed to combat this problem (Pratley 1995), however these rely on herbicides, which makes their adoption by organic farmers currently impossible. The inclusion of long legume-based pasture phases on all mixed farms may be the most effective way to combat these problems (Dalal *et al.* 1995; Grace *et al.* 1995). On the dairy farms, the negative P balance on the biodynamic farms, due to not applying P fertilisers, will eventually severely limit production. Lower yields, and lower profits, may be restricting the adoption of alternative agriculture in Australia (Wynen 1994a; Wynen 1997) and the lower economic viability of the biodynamic dairy farms may reduce the persistence of existing farms. Higher yields may also reduce long-term sustainability as they mean larger nutrient losses from the system, especially in Australia where large volumes of produce are exported overseas (see Lipsett and Dann 1983).

Table 12.9. Some characteristics of the conventional (Con.) and organic mixed farms at Ardlethan (Derrick 1996) and the NE Victorian conventional (Con.) and biodynamic (BD) dairy farms (Small *et al.* 1994a) which may influence their long-term sustainability.

	Mixed farms(1)		Dairy farms	
	Conventional	Organic	Conventional	Biodynamic
P balance	+ive	+ive (> Con.)	+ive	-ive
Erosion	?	? (2)	negligible	negligible
Energy use	Con. > Organic		?	?
Production levels	Con. > Organic		Con. > BD	
Economics	+ive	+ive	+ive	+ive (< Con.)
Irrigation water use	-	-	Con. > BD	
Pesticide use	yes	no	yes	no
VAM colonisation	low	high	low	high

(1) for an entire rotation, not just a single crop
(2) no potential to adopt minimum tillage techniques which involve herbicide use

The largest environmental problem associated with the dairy farms is their reliance on irrigation water. The biodynamic farms irrigated less frequently and, therefore, used less water than the conventional farms. However, the problems associated with irrigation, including rising water tables and salinisation on farms, along with deteriorating downstream water quality — due to factors such as eutrophication — are rapidly increasing (Mussared 1995). These may substantially reduce the lifespan of irrigation schemes (Mussared 1995). Concern is increasing over the effects of pesticides on human health (Short 1994), while the level of energy use in modern agricultural systems will require consideration as fossil fuel reserves are depleted.

Ultimately, the competing — and perhaps opposing — needs to reduce environmental degradation, feed the increasing world population and remain economically viable, are likely to result in agricultural systems which take the best aspects of current conventional and alternative systems. VAM fungi are likely to play a vital role in such agricultural systems.

12.4. Overall Project Conclusions

The conclusions are grouped into three sections: the functioning of VAM fungi on SE Australian mixed and dairy farms; general characteristics of VAM fungi; and comparisons between conventional and alternative agricultural systems.

1) VAM fungi on SE Australian mixed cereal-livestock farms and dairy farms.

• VAM colonisation levels are consistently negatively correlated with soil extractable P and plant P concentrations due to the ability of plant roots to restrict colonisation as their internal P concentration rises.

• Total soil N and plant N concentrations are positively correlated with VAM colonisation in some instances. It is possible that fungal growth is restricted by lack of N, even when plant growth is not N-limited.

• Severe drought markedly reduces VAM colonisation in annual crops. Thus, while VAM fungi may play a role in alleviating minor plant water stress, they are unlikely to reduce the effects of long-term severe water stress, especially in annual crops when colonisation is not well-established before the imposition of the water stress.

• Low VAM colonisation during severe droughts may reduce the VAM inoculum potential of the soil and could, therefore, result in an effect similar to Long Fallow Disorder in a subsequent VAM-dependent crop.

• Cereal crops on the mixed farms receive no yield benefits from VAM colonisation, while growth of subterranean clover in the annual pastures is substantially increased by VAM colonisation.

• Grasses on the dairy farms receive little net growth increase from VAM colonisation, while white clover receives a small net benefit. Compared to the mixed farms, the higher soil extractable P in the dairy pastures reduces the benefits for clover growth from VAM colonisation

• Farmers in these agricultural systems do not need to change their management practices to favour VAM fungi. Alternative farms will have close to maximum levels of colonisation in crops and pastures and only a major event, such as severe drought, will reduce VAM levels in crops. However, as no VAM dependent crops are grown, or seem likely to be grown, in the SE Australian wheatbelt, this will not cause a problem. Conventional farms would have to forgo the benefits for yields from applying soluble P fertiliser to increase VAM colonisation in crops or pasture. However, the high colonisation in the mixed farm pastures and the relatively high colonisation in the dairy

pastures means that other benefits from VAM colonisation, such as improved soil structure, will be present on conventional farms.

2) General Characteristics of VAM Fungi.

• The VAM fungi-host plant relationship may best be considered as a balance of plant energetic costs and benefits for P nutrition, but effects on uptake of other nutrients and water, and interactions with pathogens may also need to be considered.

• Costs and benefits to plants from VAM colonisation are often closely balanced, but as regulation of VAM fungi by the plant is relatively loose, minor negative balances for plants can occur. For instance, low light levels in glasshouse trials increase the costs of supporting VAM fungi, however exactly when this results in a negative effect on the plant will depend on the level of dependency of the plant.

• Results from glasshouse trials may not be particularly relevant to field conditions, due to differences in many factors including the soil environment, environmental conditions, plant growth — especially the effects of intra- and interspecific competition, grazing and root density — and the species of VAM fungi present. While simple glasshouse trials using diluted sterilised soil and single species of VAM fungi are necessary to define the potential interactions between VAM fungi and plants, more complicated trials using field soils and mimicking the conditions found in the field are necessary to gain an understanding of how VAM fungi function in the field.

• The high levels of P in many agricultural systems will increase the probability of VAM fungi acting parasitically. Conversely, the relatively low root densities and rapid growth rates in annual cropping monocultures may, if P is limiting plant growth and the crop is VAM dependent, allow VAM fungi to greatly increase crop yield.

• The percentage of root length colonised by VAM fungi is an adequate measure of VAM abundance when identifying broad relationships and trends in the field. The percentage of root length colonised by VAM fungi and colonisation intensity are consistently strongly positively correlated.

• When comparing both within and between ecosystems, the abundance of VAM fungi will be broadly negatively correlated to soil extractable P. VAM fungi may act to buffer plant P concentrations as soil P decreases. Due to the variation in the scale at which trends involving VAM fungi become clear, results from field surveys must be supported by field or glasshouse manipulative experiments to confirm which variables are responsible for the trends noted in the field.

3) Conventional and alternative agriculture.

• Due to the addition of fertilisers containing soluble P, conventional farms consistently have higher soil extractable P and significantly lower VAM colonisation levels than alternative farms. In Australia, at least on broadacre farms, no other farm management practices will have such significant consistent effects on VAM colonisation levels. VAM fungi will, therefore, contribute more towards plant nutrient uptake, and perhaps pathogen control and soil structure maintenance, on alternative farms than conventional farms. Phosphorus inputs and the levels of VAM fungi may be the most consistent, significant difference between the soil ecosystems on conventional and alternative farms in Australia.

• There is no indication that the VAM community differs between conventional and biodynamic dairy farms. VAM spore types and abundance did not differ significantly between the farms. In the glasshouse, VAM colonisation and plant growth in conventional and biodynamic dairy farm soils responded in the same manner to nutrient additions.

• Alternative farms have lower levels of *Rhizobium* nodulation in clover than conventional farms, probably due to lower plant P concentrations limiting formation of nodules.

• The soil biological interactions governing processes such as plant nutrient uptake are similar on conventional and alternative farms, but vary along a continuum — in a predictable manner — in response to quantifiable factors, such as inputs of P and organic matter. There is no indication that alternative farms function through processes not able to be defined using current scientific methods and knowledge.

• VAM fungi will play an important role in the development of more sustainable agricultural systems through their ability to influence plant nutrient uptake, nutrient cycling, soil structure and the occurrence of pathogens.

12.5 Further Research

Research into VAM fungi is currently constrained by the inability of researchers to address several fundamental issues. Finding ways to overcome these problems is essential if the functioning of VAM fungi under field conditions is to be understood. Three of these problems are listed below, followed by questions which remain to be addressed about both the role of VAM fungi in the agricultural systems sampled in this project and the role of VAM fungi in more general ecosystem processes.

1) Techniques for researching VAM fungi

• Techniques must be developed to distinguish between colonisation by different VAM species in field grown plants. Molecular techniques based on DNA sequences may prove suitable.

• Researchers need to be able to compare between mycorrhizal and non-VAM plants, without the problems associated with using sterilised soil. Development of non-VAM plant strains could be useful.

• Techniques need to be improved for indirectly investigating field systems. Glasshouse trials need to be more relevant to field conditions and indicators of VAM activity which can be measured on plants in the field need to be developed.

2) Questions remaining to be answered regarding the role of VAM fungi in SE Australian agricultural systems

• Do VAM fungi directly provide net benefits for plant growth other than through P uptake? For instance, do they enhance uptake of other nutrients or provide protection from pathogens?

• What role do VAM fungi play in the maintenance of soil structure?

• Do agricultural practices affect VAM fungi community structure on the mixed farms?

• Are VAM fungi with different life-strategies favoured under differently structured agricultural systems such as pastures and crops? If so, what are the consequences of alternating between such systems?

3) Questions remaining to be answered about the role of VAM fungi in ecosystem functioning and global-scale processes

• What factors affect the functioning of VAM fungi under field conditions? In particular, what are the influences of environmental extremes, plant density and competition, grazing of plants and other soil organisms?

• What are the primary effects on plants from VAM colonisation under field conditions? Is the ability of VAM fungi to decrease the effects of pathogens often more important than enhancing nutrient uptake? Are VAM fungi playing a major role in mediating the outcomes of plant competition and thereby influencing plant community structure and processes such as succession? Does a functional hyphal network link plants in a community and, if so, how does this influence plant growth? What indicators of plant fitness should be measured?

• How important are VAM fungi in closing nutrient cycles by reducing losses of nutrients through fixation or leaching, or in moving nutrients between dying and living plants, thereby bypassing the detritus foodweb?

• What role do VAM fungi play in global processes, such as biogeochemical cycling? Do VAM fungi need to be factored into models of these processes in order to accurately predict the consequences of large-scale human induced perturbations, such as air pollution or rising atmospheric carbon dioxide levels?

References

ABARE (1995). *Farm Surveys Report 1995*. Australian Bureau of Agricultural and Resource Economics. Canberra.

Abbott, L.K. and Robson, A.D. (1978). Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field trial. *New Phytologist,* **81**: 575-85.

Abbott, L.K. and Robson, A.D. (1979). A quantitative study of the spores and anatomy of mycorrhizas formed by a species of *Glomus*, with reference to its taxonomy. *Australian Journal of Botany,* **27**: 363-75.

Abbott, L.K. and Robson, A.D. (1981). Infectivity and effectiveness of vesicular arbuscular mycorrhizal fungi: effect of inoculum type. *Australian Journal of Agricultural Research,* **32**: 631-9.

Abbott, L.K. and Robson, A.D. (1985a). The effect of soil pH on the formation of vesicular-arbuscular mycorrhizas by two species of *Glomus*. *Australian Journal of Soil Research,* **23**: 253-61.

Abbott, L.K. and Robson, A.D. (1985b). Formation of external hyphae in soil by four species of vesicular arbuscular mycorrhizal fungi. *New Phytologist,* **99**: 245-55.

Abbott, L.K., Robson, A.D., Jasper, D.A. and Gazey, C. (1992). What is the role of VA mycorrhizal hyphae in soil. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 37-41.

Achtnich, W. and Moaward, M.A. (1986). Efficiency of vesicular-arbuscular mycorrhiza with rock phosphate as phosphorus source. In *The Importance of Biological Agriculture in a World of Diminishing Resources*, (Eds. H. Votmann, E. Boehncke and I. Fricke), Verlagsgruppe Witzenhaussen. Kassel.

Alexander, T., Meier, R., Toth, R. and Weber, H.C. (1988). Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with reference to *Zea mays* L. *New Phytologist,* **110**: 363-70.

Allen, E.B. and Allen, M.F. (1986). Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. *New Phytologist,* **104**: 559-71.

Allen, M.F. (1991). *The Ecology of Mycorrhizae*. Cambridge University Press. Cambridge.

Allen, M.F. and Boosalis, M.G. (1983). Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytologist,* **93**: 67-76.

Allen, M.F., Clouse, S.D., Weinbaum, B.S., Jeakins, S.L., Friese, C.F. and Allen, E.B. (1992). Mycorrhizae and the integration of scales: from molecules to ecosystems. In *Mycorrhizal Functioning*, (Ed. M.J. Allen), Chapman and Hall. New York. 488-516.

Allsopp, N. and Stock, W.D. (1992). Density dependent interactions between VA mycorrhizal fungi and even-aged seedlings of two perennial Fabaceae species. *Oecologia,* **91**: 281-7.

Allsopp, N. and Stock, W.D. (1994). VA mycorrhizal infection in relation to edaphic characteristics and disturbance regime in three lowland plant communities in the south-western Cape, South Africa. *Journal of Ecology,* **82**: 271-9.

Allsopp, N. and Stock, W.D. (1995). Relationships between seed reserves, seedling growth and mycorrhizal responses in 14 related shrubs (Rosidae) from a low-nutrient environment. *Functional Ecology,* **9**: 248-54.

Amijee, F., Stribley, D.P. and Lane, P.W. (1993a). The susceptibility of roots to infection by an arbuscular mycorrhizal fungus in relation to age and phosphorus supply. *New Phytologist,* **125**: 581-6.

Amijee, F., Stribley, D.P. and Tinker, P.B. (1993b). The development of endomycorrhizal root systems. VIII Effects of soil phosphorus and fungal colonisation on the concentration of soluble carbohydrates in roots. *New Phytologist,* **123**: 297-306.

AQIS (1997). *National Standard for Organic and Biodynamic Produce.* Canberra.

Armstrong, R.D., Helgar, K.R. and Christie, E.K. (1992). Vesicular-arbuscular mycorrhiza in semi-arid pastures of south-east Queensland and their effect on growth responses to phosphorus fertilisers by grasses. *Australian Journal of Agricultural Research,* **43**: 1143-55.

Auge, R.M., Schekel, K.A. and Wample, R.L. (1986). Osmotic adjustment in leaves of VA mycorrhizal and nonmycorrhizal rose plants in response to drought stress. *Plant Physiology,* **82**: 765-70.

Azcón, R., Gomez, M. and Tobar, R. (1992). Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and phosphorus-fertilised plants of *Lactuca sativa* L. *New Phytologist,* **121**: 227-34.

Aziz, T., Habte, M. and Yuen, J.E. (1991). Inhibition of mycorrhizal symbiosis in *Leucaena leucocephala* by chlorothalonil. *Plant and Soil,* **131**: 47-52.

Bååth, E. and Hayman, D.S. (1984). Effect of soil volume and plant density on mycorrhizal infection and growth response. *Plant and Soil,* **77**: 373-6.

Bååth, E. and Spokes, J. (1989). The effect of added nitrogen and phosphorus on mycorrhizal growth response and infection in *Allium schoenoprasum. Canadian Journal of Botany,* **67**: 3227-32.

Baon, J.B., Smith, S.E. and Alston, A.M. (1993a). Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant and Soil,* **157**: 97-106.

Baon, J.B., Smith, S.E. and Alston, A.M. (1993b). Phosphorus allocation in P-efficient and inefficient barley cultivars as affected by mycorrhizal infection. *Plant and Soil,* **155/156**: 277-80.

Baon, J.B., Smith, S.E., Alston, A.M. and Wheeler, R.D. (1992). Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. *Australian Journal of Agricultural Research,* **43**: 479-91.

Barea, J.M., Azcón-Aguilar, C. and Azcón, R. (1987). Vesicular-arbuscular mycorrhiza improve both symbiotic N_2 fixation and N uptake from soil as assessed with a ^{15}N technique under field conditions. *New Phytologist,* **106**: 717-25.

Baylis, G.T.S. (1975). The Magnolioid mycorrhiza and mycotrophy in root systems derived from it. In *Endomycorrhizas*, (Eds. F.E. Sanders, B. Mosse and P.B. Tinker), Academic Press. London. 373-89.

Bethlenfalvay, G.F. and Schüepp, H. (1994). Arbuscular mycorrhizas and agrosystem stability. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Systems*, (Eds. S. Gianinazzi and H. Schüepp), Birkhäuser Verlag. Basel. 117-31.

Bethlenfalvay, G.J. and Barea, J. (1994). Mycorrhizae in sustainable agriculture. I. effects on seed yield and soil aggregation. *American Journal of Experimental Agriculture,* **9**: 157-61.

Bethlenfalvay, G.J. and Pacovsky, R.S. (1983). Light effects in mycorrhizal soybeans. *Plant Physiology,* **73**: 969-72.

Bethlenfalvay, G.J., Brown, M.S. and Pacovsky, R.S. (1982). Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: development of host plant. *Phytopathology,* **72**: 889-93.

Bethlenfalvay, G.J., Brown, M.S., Ames, R.N. and Thomas, R.S. (1988). Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Physiologia Plantarum,* **72**: 565-71.

Black, J.N. (1957). Seed size as a factor in the growth of subterranean clover (*Trifolium subterranean* L.) under spaced and sward conditions. *Australian Journal of Agricultural Research,* **8**: 335-51.

Bloss, H.E. and Pfeiffer, C.M. (1984). Latex content and biomass increase in mycorrhizal guayule (*Parthenium argentatum*) under field conditions. *Annals of Applied Biology,* **104**: 175-83.

Bokhorst, J.G. (1989). The organic farm at Nagele. In *Development of Farming Systems*, (Ed. Zadoks), Pudoc. Wageningen.

Bolan, N.S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil,* **134**: 189-207.

Bolan, N.S. and Robson, A.D. (1983). Plant and soil factors including mycorrhizal infection causing sigmoidal response of plants to applied phosphorus. *Plant and Soil,* **73**: 187-201.

Bolan, N.S., Robson, A.D. and Barrow, N.J. (1984). Increasing phosphorus supply can increase the infection of plant roots by vesicular-arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry,* **16**: 419-20.

Bolan, N.S., White, R.E. and Hedley, M.J. (1990). A review of the use of phosphate rocks as fertilisers for direct application in Australia and New Zealand. *Australian Journal of Experimental Agriculture,* **30**: 297-313.

Braunberger, P.G., Abbott, L.K. and Robson, A.D. (1994). The effect of rain in the dry season on the formation of vesicular-arbuscular mycorrhizas in the growing season of annual clover-based pastures. *New Phytologist,* **127**: 107-14.

Braunberger, P.G., Abbott, L.K. and Robson, A.D. (1997). Early vesicular-arbuscular mycorrhizal colonisation in soil collected from an annual clover-based pasture in a Mediterranean environment: soil temperature and the timing of autumn rains. *Australian Journal of Agricultural Research,* **48**: 103-10.

Bruce, A., Smith, S.E. and Tester, M. (1994). The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. *New Phytologist,* **127**: 507-14.

Brundrett, M. (1991). Mycorrhizas in natural ecosystems. *Advances in Ecological Research,* **21**: 171-312.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). *Working with Mycorrhizas in Forestry and Agriculture.* Australian Centre for International Agricultural Research. Canberra.

Brundrett, M.C. and Abbott, L.K. (1994). Mycorrhizal fungus propagules in the jarrah forest I: seasonal study of inoculum levels. *New Phytologist,* **127**: 539-46.

Brundrett, M.C., Ashwath, N. and Jasper, D.A. (1996). Mycorrhizas in the Kakadu region of tropical Australia I. Propagules of mycorrhizal fungi and soil properties in natural habitats. *Plant and Soil,* **184**: 159-71.

Buwalda, J.G. and Goh, K.M. (1982). Host fungus competition for carbon as a cause of growth depression in vesicular-arbuscular mycorrhizal ryegrass. *Soil Biology and Biochemistry,* **14**: 103-6.

Buwalda, J.G., Stribley, D.P. and Tinker, P.B. (1985a). Effects of vesicular-arbuscular mycorrhizal infection in first, second and third cereal crops. *Journal of Agricultural Science, Cambridge,* **105**: 631-47.

Buwalda, J.G., Stribley, D.P. and Tinker, P.B. (1985b). Vesicular-arbuscular mycorrhizas of winter and spring cereals. *Journal of Agricultural Science,* **105**: 649-59.

Cade-Menun, B.J., Berch, S.M. and Bomke, A.A. (1991). Seasonal colonisation on winter wheat in South Coastal British Columbia by vesicular-arbuscular mycorrhizal fungi. *Canadian Journal of Botany,* **69**: 78-86.

Campbell, K.O. and Bowyer, J.W. (1990). *The Scientific Basis of Modern Agriculture.* Sydney University Press. Melbourne.

Clapp, J.P., Young, J.P.W., Merryweather, J.M. and Fitter, A.H. (1995). Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist,* **130**: 2590-265.

Cock, S. (1991). *A comparison of soil and plant root characteristics in irrigated summer pasture from two different farming systems.* Bachelor of Agricultural Science Thesis. La Trobe University, School of Agriculture.

Colwell, J.D. (1963). The estimation of the phosphorus fertiliser requirements of wheat in southern NSW by soil analysis. *Australian Journal of Agriculture and Animal Husbandry,* **3**: 190-7.

Conacher, A. and Conacher, J. (1995). *Rural Land Degradation in Australia.* Oxford University Press. Melbourne.

Cook, P.J. (1982). World availability of phosphorus: an Australian perspective. In *Phosphorus in Australia,* (Eds. A.B. Costin and C.H. Williams), Centre for Resource and Environmental Studies, Australian National University. Canberra. 1-41.

Cooper, K. (1978). Adaptation of mycorrhizal fungi to phosphate fertilisers. In *Plant Nutrition 1978: Proceedings of the 8th international colloquium on plant analysis and fertiliser problems,* (Eds. A.R. Ferguson, R.L. Bieleski and I.B. Ferguson). Auckland. 107.

Cornish, P.S. and Murray, G.M. (1989). Low rainfall rarely limits wheat yields in southern NSW. *Australian Journal of Experimental Agriculture,* **29**: 77-83.

Coventry, D.R., Hirth, J.R. and Reeves, T.G. (1985). Development of populations of *Rhizobium trifolii* and nodulation of subterranean clover following the cropping phase in crop-pasture rotations in southeastern Australia. *Soil Biology and Biochemistry,* **17**: 17-22.

Crush, J.R. (1995). Effect of VA mycorrhizas on phosphorus uptake and growth of white clover (*Trifolium repens* L.) growing in association with ryegrass (*Lolium perenne* L.). *New Zealand Journal of Agricultural Research,* **38**: 303-7.

Cuenca, G. and Meneses, E. (1996). Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil,* **183**: 315-22.

Daft, M.J. and El-Giahmi, A.A. (1978). Effects of arbuscular mycorrhiza on plant growth VIII. Effects of defoliation and light on selected hosts. *New Phytologist,* **80**: 365-72.

Dalal, R.C., Strong, W.M., Weston, E.J., Cooper, J.E., Lehane, K.J., King, A.J. and Chicken, C.J. (1995). Sustaining productivity of a Vertisol at Warra, Queensland, with fertilisers, no-tillage, or legumes 1. organic matter status. *Australian Journal of Experimental Agriculture,* **35**: 903-14.

Daniels Hetrick, B.A., Hetrick, J.A. and Bloom, J. (1984). Interaction of mycorrhizal infection, phosphorus level, and moisture stress in growth of field corn. *Canadian Journal of Botany,* **62**: 2267-71.

Daniels Hetrick, B.A., Thompson Wilson, G., Gerschefske Kitt, D. and Schwab, A.P. (1988). Effects of soil microorgansims on mycorrhizal contribution to growth of big bluestem grass in non-sterile soil. *Soil Biology and Biochemistry,* **20**: 501-7.

Dann, P.R., Derrick, J.W., Dumaresq, D.C. and Ryan, M.H. (1996). The response of organic and conventionally grown wheat to superphosphate and reactive rock phosphate. *Australian Journal of Experimental Agriculture,* **36**: 71-8.

Davidson, B.R. (1969). *Australia: Wet or Dry?* Melbourne University Press. Melbourne.

Davidson, B.R. (1990). Agriculture and the Economy. In *The Manual of Australian Agriculture*, (Ed. R.L. Reid), Butterworths. Sydney. 1-19.

Davidson, S. (1986). Cultivation and soil organic matter. *Rural Research,* **131**: 13-8.

De Marco, D.G. (1990). Effect of seed weight, and seed phosphorus and nitrogen concentrations on the early growth of wheat seedlings. *Australian Journal of Experimental Agriculture,* **30**: 545-9.

Dehn, B., Bodmer, M. and Schüepp, H. (1990). Influence of herbicides on VA mycorrhizal propagation in soil. *Symbiosis,* **9**: 223-7.

Dehn, H.W. (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology,* **72**: 1115-9.

Derrick, J. (1994). Energy use and efficiency in organic and conventional broadacre cereal livestock production systems. In *1991 International Federation of Organic Agriculture Movements Conference*, Lincoln University. New Zealand.

Derrick, J. and Ryan, M. (1998). Influence of seed phosphorus content on seedling growth in wheat: implications for organic and conventional farm management in south east Australia. *Biological Agriculture and Horticulture,* in press.

Derrick, J.W. (1996). *A comparison of agroecosystems: organic and conventional broadacre farming in south east Australia.* PhD thesis, Australian National University.

Dodd, J.C. and Jeffries, P. (1986). Early development of vesicular-arbuscular mycorrhizas in autumn-sown cereals. *Soil Biology and Biochemistry,* **18**: 149-54.

Douds, D., Nagahashi, J. and Abney, G. (1996). Phosphorus amendment inhibits hyphal branching of the AM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 44.

Douds, D.D., Galvez, L., Janke, R.R. and Wagoner, P. (1995). Effect of tillage and farming system upon populations and distribution of vesicular arbuscular mycorrhizal fungi. *Agriculture, Ecosystems and Environment,* **52**: 111-8.

Doyle, P.T., Stockdale, C.R. and Lawson, A.R. (1996). *Pastures for Dairy Production in Victoria*. Agriculture Victoria. Tatura.

Dumaresq, D., Greene, R. and van Kerkhoff, L. (Eds.). (1997). *Organic Agriculture in Australia. Proceedings of the national symposium on organic agriculture: research and development 30 June - 3 July 1996.* RIRDC Research Paper No 97/14: Canberra.

Dumsday, R., Edwards, G. and Chisholm, A. (1990). Resource Management. In *Agriculture in the Australian Economy*, (Ed. D.B. Williams), Sydney University Press. Sydney. 172-86.

Dunne, M.J. and Fitter, A.H. (1989). The phosphorus budget of a field-grown strawberry (*Fragaria x ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution. *Annals of Applied Biology,* **114**: 185-93.

Edathil, T.T., Manian, S. and Udaiyan, K. (1996). Interaction of multiple VAM fungal species on root colonisation, plant growth and nutrient status of tomato seedlings (*Lycopersicon esculentum* Mill.). *Agriculture, Ecosystems and Environment,* **59**: 63-8.

Ellis, J.R., Larsen, H.J. and Boosalis, M.G. (1985). Drought resistance of wheat plants inoculated with vesicular-arbuscular mycorrhizae. *Plant and Soil,* **86**: 369-78.

Ellis, J.R., Roder, W. and Mason, S.C. (1992). Grain sorghum - soybean rotation and fertilisation: influence on vesicular-arbuscular mycorrhizal fungi. *Soil Science Society of America Journal,* **56**: 789-94.

Elmholt, S. and Kjøller, A. (1989). Comparison of the occurrence of the saprophytic soil fungi in two differently cultivated field surveys. *Biological Agriculture and Horticulture,* **6**: 229-39.

Ericsson, T. (1995). Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil,* **168-169**: 205-14.

Evans, D.G. and Miller, M.H. (1988). Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize. I Causal relations. *New Phytologist,* **110**: 67-74.

Evans, D.G. and Miller, M.H. (1990). The role of the external mycelial network in the effect of soil disturbance upon vesicular-arbuscular colonisation of maize. *New Phytologist,* **114**: 65-71.

Evans, J., Dear, B. and O'Connor, G.E. (1990). Influence of an acid soil on the herbage yield and nodulation of five annual pasture legumes. *Australian Journal of Experimental Agriculture,* **30**: 55-60.

Fettell, N. (1980). Higher yields from long fallow in the central west. *Agricultural Gazette of NSW,* **91**: 22-4.

Fitter, A.H. (1977). Influence of mycorrhizal infection on competition for phosphorus and potassium by two grasses. *New Phytologist,* **79**: 119-25.

Fitter, A.H. (1985). Functioning of vesicular-arbuscular mycorrhizas under field conditions. *New Phytologist,* **99**: 257-65.

Fitter, A.H. (1986). Effect of Benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. *New Phytologist,* **103**: 767-76.

Fitter, A.H. (1988). Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. *Journal of Experimental Botany,* **39**: 595-603.

Fitter, A.H. and Merryweather, J.W. (1992). Why are some plants more mycorrhizal than others? An ecological inquiry. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 26-36.

Föhse, D., Claasen, N. and Jungk, A. (1991). Phosphorus efficiency of plants II significance of root radius, root hairs and cation-anion balance for phosphorus influx in seven plant species. *Plant and Soil,* **132**: 261-72.

Francis, R. and Read, D.J. (1994). The contribution of mycorrhizal fungi to the determination of plant community structure. *Plant and Soil,* **159**: 11-25.

Galvez, L., Douds, D.D., Wagoner, P., Longnecker, R.L., Drinkwater, L.E. and Janke, R.R. (1995). An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. *American Journal of Alternative Agriculture,* **10**: 152-6.

Gange, A.C. and Brown, V.K. (1992). Interactions between soil-dwelling insects and mycorrhizas during early plant succession. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 177-82.

Gange, A.C. and Nice, E.N. (1997). Performance of the thistle gall fly, *Urophora cardui*, in relation to host plant nitrogen and mycorrhizal colonisation. *New Phytologist,* **137**: 335-43.

Gange, A.C. and West, H.M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects on *Plantago lanceolata* L. *New Phytologist,* **128**: 79-87.

Gatehouse, R. (1995). *Mycorrhizae, soil management and soil structure in a neighbouring organic and conventional dryland wheat farm at Ardlethan, NSW.* Science (Honours) Thesis, Australian National University.

Gavito, M.E. and Varela, L. (1993). Seasonal dynamics of mycorrhizal associations in maize fields under low input agriculture. *Agriculture, Ecosystems and Environment,* **45**: 275-82.

Gavito, M.E. and Varela, L. (1995). Response of "criollo" maize to single and mixed species inocula of arbuscular mycorrhizal fungi. *Plant and Soil,* **176**: 101-5.

Gemma, J.N. and Koske, R.E. (1992). Are mycorrhizal fungi present in early stages of primary succession. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAM International. Wallingford. 183-9.

Gemma, J.N., Koske, R.E. and Carreiro, M. (1989). Seasonal dynamics of selected species of VA-mycorrhizal fungi in a sand dune. *Mycological Research,* **92**: 317-21.

Gerdemann, J.W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Phytopathology,* **6**: 397-418.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring VAM infection in roots. *New Phytologist,* **84**: 489-500.

Grace, C. and Stribley, D.P. (1991). A safer procedure for routine staining of vesicular arbuscular mycorrhizal fungi. *Mycological Research,* **95**: 1160-2.

Graham, J.H. and Menge, J.A. (1982). Influence of VAM and soil phosphorus on take-all disease of wheat. *Phytopathology,* **72**: 95-8.

Graham, J.H., Leonard, R.T. and Menge, J.A. (1981). Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiology,* **68**: 548-52.

Graham, J.H., Linderman, R.G. and Menge, J.A. (1982). Development of external hyphae of different isolates of mycorrhizal *Glomus* spp. in relation to root colonisation and growth of troyer citrange. *New Phytologist,* **91**: 183-9.

Grime, J.P., Mackey, J.M.L., Hillier, S.H. and Read, D.J. (1987). Floristic diversity in a model system using experimental microcosms. *Nature,* **328**: 420-2.

Groffman, P.M. (1996). Integration of soil science in ecological research. In *The Role of Soil Science in Interdisciplinary Research*, (Eds. R.J. Wagenet and J. Bouma), Soil Science Society of America. Madison. 57-65.

Gruhn, C.M. (1996). The arbuscular mycorrhizal status of vegetable crops grown on an organic farm. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 55.

Grace, P.R., Oades, J.M., Keith, H. and Hancock, T.W. (1995). Trends in wheat yields and soil organic carbon in the Permanent Rotation Trial at the Waite Agricultural Research Institute, South Australia. *Australian Journal of Experimental Agriculture,* **35**: 857-64.

Guo, B.Z., Hendrix, J.W., An, Z.-Q. and Ferriss, R.S. (1992). Role of *Acremonium* endophyte of fescue on inhibition of colonisation and reproduction of mycorrhizal fungi. *Mycologia,* **84**: 882-5.

Gupta, V.V.S.R. and Germida, J.J. (1988). Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology and Biochemistry,* **20**: 777-86.

Hahn, A., Gianinazzi-Pearson, V. and Hock, B. (1994). Characterization of arbuscular mycorrhizal fungi by immunochemical methods. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, (Eds. S. Gianinazzi and H. Schüepp), Birkhäuser Verlag. Basel. 25-39.

Hall, I.R. (1978). Effects of endomycorrhizas on the competitive ability of white clover. *New Zealand Journal of Agricultural Research,* **21**: 509-15.

Hamblin, A. (1991). *Sustainability: Physical and Biological Considerations for Australian Environments.* Bureau of Rural Resources. Canberra.

Hamblin, A. and Kyneur, G. (1993). *Trends in Wheat Yields and Soil Fertility in Australia*. Department of Primary Industries and Energy, Bureau of Resource Sciences, Australian Government Publishing Service. Canberra.

Hamblin, A., Tennant, D. and Perry, M.W. (1990). The cost of stress: dry matter partitioning changes with seasonal supply of water and nitrogen to dryland wheat. *Plant and Soil,* **122**: 47-58.

Hamel, C., Fyles, H. and Smith, D.L. (1990). Measurement of development of endomycorrhizal mycelium using three different vital stains. *New Phytologist,* **115**: 297-302.

Hassall and Associates (1996). *The Domestic Market for Australian Organic Produce: An Update*. RIRDC. Canberra.

Hayman, D.S. and Mosse, B. (1979). Improved growth of white clover in hill grasslands by mycorrhizal inoculation. *Annals of Applied Biology,* **93**: 141-8.

Hayman, D.S. and Stovold, G.E. (1979). Spore populations and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales. *Australian Journal of Botany,* **27**: 227-33.

Haynes, R.J. and Williams, P.H. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy,* **49**: 119-99.

Heffernan, B. (1985a). Determination of phosphorus and nitrogen in foliage by automated spectrophotometric method. In *A Handbook of Methods of Inorganic Chemical Analysis for Forest Soils, Foliage and Water*, CSIRO Division of Forest Research. Canberra. 49-53.

Heffernan, B. (1985b). Determination of phosphorus and nitrogen in soil by automated spectrophotometric method. In *A Handbook of Methods of Inorganic Chemical Analysis for Forest Soils, Foliage and Water*, (Ed. B. Heffernan), CSIRO Division of Forest Research. Canberra.

Hendrix, J.W. (1985). Tobacco stunt, a disease of burley tobacco controlled by soil fumigants. *Plant Disease,* **69**: 445-7.

Hendrix, J.W., Guo, B.Z. and An, Z.-Q. (1995). Divergence of mycorrhizal fungal communities in crop production systems. *Plant and Soil,* **170**: 131-40.

Holford, I.C.R. (1997). Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research,* **35**: 227-39.

Holford, I.C.R. and Doyle, A.D. (1992). Influence of intensity/quantity characteristics of soil phosphorus tests on their relationships to phosphorus responsiveness of wheat under field conditions. *Soil Fertility and Plant Nutrition,* **30**: 343-56.

Jakobsen (1992). Phosphorus transport by external hyphae of vesicular-arbuscular mycorrhizal fungi. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 48-54.

Jakobsen, I. (1994). Research approaches to study the functioning of vesicular-arbuscular mycorrhizas in the field. *Plant and Soil,* **159**: 141-7.

Jakobsen, I. (1996). Competition for soil P between roots and hyphae of arbuscular mycorrhizas. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 65.

Jakobsen, I. and Neilson, N.E. (1983). Vesicular-arbuscular mycorrhiza in field grown crops. I. mycorrhizal infection in cereals and peas at various times and soil depths. *New Phytologist,* **93**: 401-13.

Jakobsen, I. and Rosendahl, L. (1990). Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist,* **115**: 77-83.

Janke, R.R., Mt Pleasant, J., Peters, S.E. and Böhlke, M. (1991). Long-term, low-input cropping systems research. In *Sustainable Agriculture Research and Education in the Field*, (Eds. B. Rice and J.P. Madden). National Academy Press. Washington DC. 291-317.

Jasper, D.A., Abbott, L.K. and Robson, A.D. (1989). Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* **112**: 93-9.

Jasper, D.A., Abbott, L.K. and Robson, A.D. (1991). The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. *New Phytologist,* **118**: 471-6.

Jasper, D.A., Abbott, L.K. and Robson, A.D. (1993). The survival of infective hyphae of vesicular-arbuscular mycorrhizal fungi in dry soil: an interaction with sporulation. *New Phytologist,* **124**: 473-9.

Jasper, D.A., Robson, A.D. and Abbott, L.K. (1979). Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biology and Biochemistry,* **11**: 501-5.

Jayachandran, K. and Schwab, A.P. (1992). Mineralisation of organic phosphorus by vesicular-arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry,* **24**: 897-903.

Jeffries, P. and Barea, J.M. (1994). Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systems. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Systems*, (Eds. S. Gianinazzi and H. Schüepp), Birkhäuser Verlag. Basel. 101-15.

Jensen, A. (1983). The effect of indigenous vesicular-arbuscular mycorrhizal fungi on nutrient uptake and growth of barley in two Danish soils. *Plant and Soil,* **70**: 155-63.

Jensen, A. and Jakobsen, I. (1980). The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertiliser treatments. *Plant and Soil,* **55**: 403-14.

Johnson, N.C. (1993). Can fertilisation of soil select less mutualistic mycorrhizae. *Ecological Applications,* **3**: 749-57.

Johnson, N.C., Copeland, P.J., Crookston, R.K. and Pfleger, F.L. (1992). Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal,* **84**: 387-90.

Joner, E.J. and Jakobsen, I. (1995). Uptake of ^{32}P from labelled organic matter by mycorrhizal and non-mycorrhizal subterranean clover (*Trifolium subterraneum* L.). *Plant and Soil,* **172**: 221-7.

Jones, K. and Hendrix, J.W. (1987). Inhibition of root extension in tobacco by the mycorrhizal fungus *Glomus macrocarpum* and its prevention by benomyl. *Soil Biology and Biochemistry,* **19**: 297-9.

Kabir, Z., Ohalloran, I.P., Fyles, J.W. and Hamel, C. (1997). Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization - hyphal density and mycorrhizal root colonisation. *Plant and Soil,* **192**: 285-93.

Khan, A.G. (1975). The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals II effects on wheat. *Annals of Applied Biology,* **80**: 27-36.

Klironomos, J.N. and Kendrick, W.B. (1993). Research on mycorrhizas: trends in the past 40 years as expressed in the 'MYCOLIT' database. *New Phytologist,* **125**: 595-600.

Kohn, G.D., Storrier, R.R. and Cuthbertson, E.G. (1966). Fallowing and wheat production in southern N.S.W. *Australian Journal of Experimental Agriculture and Animal Husbandry,* **6**: 233-41.

Koide, R. (1985). The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. *New Phytologist,* **99**: 449-62.

Koide, R.T. (1991). Density-dependent response to mycorrhizal infection in *Abutilon theophrasti* Medic. *Oecologia,* **85**: 389-95.

Koide, R.T. and Li, M. (1989). Appropriate controls for vesicular-arbuscular mycorrhiza research. *New Phytologist,* **111**: 35-44.

Koide, R., Li, M., Lewis, J. and Irby, C. (1988). Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. I Wild vs cultivated oats. *Oecologia,* **77**: 537-43.

Kolisko, E. and Kolisko, L. (1978). *Agriculture of Tomorrow.* Kolisko Archive Publications. Bournemouth.

Kough, J.L., Gianinazzi-Pearson, V. and Gianinazzi, S. (1987). Depressed metabolic activity of vesicular-arbuscular fungi after fungicide applications. *New Phytologist,* **106**: 707-15.

Kucey, R.M.N. and Paul, E.A. (1982). Carbon flow, photosynthesis, and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). *Soil Biology and Biochemistry,* **14**: 407-12.

Kumar, K., Prihar, S.S. and Gajri, P.R. (1993). Determination of root distribution of wheat by auger sampling. *Plant and Soil,* **149**: 245-53.

La Rooj, M. (1989). Soil fertility. In *Biodynamics: new directions for farming and gardening in New Zealand*, Random House. Auckland. 18-24.

Ledgard, S.F. and Steele, K.W. (1992). Biological nitrogen fixation in mixed legume/grass pastures. In *Biological Nitrogen Fixation for Sustainable Agriculture*, (Eds. J.K. Ladha, T. George and B.B. Bohlool), Kluwer. Dordrecht. 137-53.

Leeper, G.W. and Uren, N.C. (1993). *Soil Science: An Introduction.* Melbourne University Press. Melbourne.

Lengnick, L.L. and King, L.D. (1986). Comparison of the phosphorus status of soils managed organically and conventionally. *American Journal of Alternative Agriculture,* **1**: 108-14.

Linderman, R.G. (1992). Vesicular-arbuscular mycorrhizae and soil microbial interactions. In *Mycorrhizae in Sustainable Agriculture*, (Eds. G.J. Bethlenfalvay and R.G. Linderman), American Society of Agronomy. Madison. 45-70.

Lindsay, A.M. (1985). Are Australian Soils Different? In *Are Australian Ecosystems Different?*, (Eds. J.R. Dodson and M. Westoby), Proceedings of the Ecological Society of Australia Vol. 14. Sydney. 83-97.

Lipsett, J. and Dann, P.R. (1983). Wheat: Australia's hidden mineral export. *The Journal of the Australian Institute of Agricultural Science,* **49**: 81-9.

Lockeretz, W., Shearer, G., Sweeney, S., Kuepper, G., Wanner, D. and Kohl, D.H. (1980). Maize yields and soil nutrient levels with and without pesticides and standard commercial fertilizers. *Agronomy Journal,* **72**: 65-72.

Lopez-Real, J.M. (1985). Sustainable agriculture: the microbial potential- the microbiologists challenge. In *The Role of Micro-Organisms in a Sustainable Agriculture*, (Eds. J.M. Lopez-Real and R.D. Hodges), Academic. Berkhamstead.

Lu, S., Braunberger, P.G. and Miller, M.H. (1994). Response of vesicular-arbuscular mycorrhizas of maize to various rates of P addition to different rooting zones. *Plant and Soil,* **158**: 119-28.

Lu, X. and Koide, R.T. (1994). The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist,* **128**: 211-8.

Lytton-Hitchins, J.A. (1992). *A comparison of the physical and chemical soil properties of adjacent bio-dynamic and conventionally managed paddocks in N.E. Victoria.* Honours thesis, School of Crop Sciences, The University of Sydney.

Lytton-Hitchins, J.A., Koppi, A.J. and McBratney, A.B. (1994). The soil condition of adjacent bio-dynamic and conventionally managed dairy pastures in Victoria, Australia. *Soil Use and Management,* **10**: 79-87.

Macgregor, A.N. (1994). Beneficial soil biota in organic and alternative farming systems. In *Soil Biota: Management in Sustainable Farming Systems*, (Eds. C.E. Pankhurst, B.M. Doube, V.V.S.R. Gupta and P.R. Grace). CSIRO. Adelaide. 204-8.

Madge, D.G. (1995). *Organic Agriculture: Getting Started.* Agmedia. Melbourne.

Manske, G.G.B. (1989). Genetical Analysis of the efficiency of VA mycorrhiza with spring wheat. *Agriculture, Ecosystems and Environment,* **29**: 273-80.

Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil,* **159**: 89-102.

Mårtensson, A.M. and Carlgren, K. (1994). Impact of phosphorus fertilisation on VAM diaspores in two Swedish longterm field experiment. *Agriculture, Ecosystems, Environment,* **47**: 327-34.

Martins, M.A. (1992). The role of the external mycelial network of vesicular arbuscular mycorrhizal fungi: a study of carbon transfer between plants interconnected by a common mycelium. *Mycorrhiza,* **2**: 69-73.

McDonald, J.W., Small, D.R. and Wales, W. (1994). Health, management and nutrition of dairy cattle on biodynamic farms in south-eastern Australia. In *10th International Organic Agriculture IFOAM Conference*, Lincoln University, New Zealand.

McGonigle, T.P. and Fitter, A.H. (1988). Growth and phosphorus inflows of *Trifolium repens* L. with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. *New Phytologist,* **108**: 59-65.

McGonigle, T.P. and Fitter, A.H. (1990). Ecological specificity of vesicular-arbuscular mycorrhizal associations. *Mycological Research,* **94**: 120-2.

McLaughlin, M.J., Alston, A.M. and Martin, J.K. (1988). Phosphorus cycling in wheat pasture rotation I -the source of the phosphorus. *Australian Journal of Soil Research,* **26**: 323-31.

McLennan, W. (1996). *Australian Agriculture and the Environment.* Australian Bureau of Statistics. Canberra.

McNaughton, S.J. and Oesterheld, M. (1990). Extramatrical mycorrhizal abundance and grass nutrition in a tropical grazing ecosystem, the Serengeti National Park, Tanzania. *Oikos,* **59**: 92-6.

Menge, J.A. (1982). Effect of soil fumigants and fungicides on vesicular-arbuscular mycorrhizal fungi. *Phytopathology,* **72**: 1125-32.

Merryweather, J. and Fitter, A. (1995a). Arbuscular mycorrhiza and phosphorus as controlling factors in the life history of *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. *New Phytologist,* **129**: 629-36.

Merryweather, J. and Fitter, A. (1995b). Phosphorus and carbon budgets: mycorrhizal contribution in *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. under natural conditions. *New Phytologist,* **129**: 619-27.

Meyer, J.R. and Linderman, R.G. (1986). Selective influence on populations of rhizosphere bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum. Soil Biology and Biochemistry,* **18**: 191-6.

Miller, M.H. and McGonigle, T.P. (1992). Soil disturbance and the effectiveness of arbuscular mycorrhizas in an agricultural ecosystem. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 156-63.

Miller, R. and Jackson, L. (1996). On farm survey of mycorrhizae in lettuce roots. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 87.

Miller, R.M. and Jastrow, J.D. (1990). Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry,* **22**: 579-84.

Miller, R.M. and Jastrow, J.D. (1992a). Extraradical hyphal development of vesicular-arbuscular mycorrhizal fungi in a chronosequence of prairie restorations. In *Mycorrhizas in Ecosystems*, (Eds. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), CAB International. Wallingford. 171-6.

Miller, R.M. and Jastrow, J.D. (1992b). The role of mycorrhizal fungi in soil conservation. In *Mycorrhizae in Sustainable Agriculture*, (Eds. G.J. Bethlenfalvay and R.G. Linderman), American Society of Agronomy. Madison. 29-44.

Miller, R.M. and Jastrow, J.D. (1994). Vesicular-arbuscular mycorrhizae and biogeochemical cycling. In *Mycorrhizae and Plant Health*, (Eds. F.L. Pfleger and R.G. Linderman), APS Press. St. Paul. 189-212.

Mohammad, M.J., Pan, W.L. and Kennedy, A.C. (1995). Wheat responses to vesicular-arbuscular fungal inoculation of soils from eroded toposequence. *Soil Science Society of America Journal,* **59**: 1086-90.

Mosse, B., Powell, C.L. and Hayman, D.S. (1976a). Plant growth responses to vesicular-arbuscular mycorrhiza. IX Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. *New Phytologist,* **76**: 331-42.

Mosse, B., Powell, C.L. and Hayman, D.S. (1976b). Plant growth responses to vesicular-arbuscular mycorrhizas. *New Phytologist,* **76**: 331-42.

Mullen, C.L. and Dellow, J.J. (1995). *Weed Control in Winter Crops 1995.* NSW Agriculture. Orange.

Murphy, B.W. and Harte, A.J. (1992). Stabilising soils with pasture. In *Sustainable Grasslands Management of NSW: Seventh Annual Conference of the Grasslands Society of NSW,* Queanbeyan. 14-21.

Mussared, D. (1995). Irrigation at the crossroads. *ECOS,* **85**: 17-8.

Nadian, H., Smith, S.E., Alston, A.M. and Murray, R.S. (1996). The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonisation. *Plant and Soil,* **182**: 39-49.

National Research Council (1989). *Alternative Agriculture.* National Academy Press. Washington, D.C.

Nelsen, C.E. and Safir, G.R. (1982). Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. *Planta,* **154**: 407-13.

Newbould, P. and Rangeley, A. (1984). Effect of lime, phosphorus and mycorrhizal fungi on growth, nodulation and nitrogen fixation by white clover (*Trifolium repens*) grown in UK hill soils. *Plant and Soil,* **76**: 105-14.

Newman, E.I. (1988). Mycorrhizal links between plants: their functioning and ecological significance. *Advances in Ecological Research,* **18**: 243-70.

Newman, E.I. and Andrews, R.E. (1973). Uptake of phosphorus and potassium in relation to root growth and density. *Plant and Soil,* **38**: 49-69.

Newman, E.I. and Eason, W.R. (1989). Cycling of nutrients from dying roots to living plants, including the role of mycorrhizas. In *Ecology of Arable Land,* (Eds. M. Clarholm and L. Bergström), Kluwer Academic. 133-7.

Newman, E.I. and Reddell, P. (1987). The distribution of mycorrhizas among families of vascular plants. *New Phytologist,* **106**: 745-51.

Newsham, K.K., Fitter, A.H. and Watkinson, A.R. (1995). Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology,* **83**: 991-1000.

Nguyen, M.L. and Haynes, R.J. (1995). Energy and labour efficiency for three pairs of conventional and alternative mixed cropping (pasture-arable) farms in Canterbury, New Zealand. *Agriculture, Ecosystems and Environment,* **52**: 163-72.

Nicolson, T.H. (1975). Evolution of Vesicular-Arbuscular Mycorrhizas. In *Endomycorrhizas,* (Eds. F.E. Sanders, B. Mosse and P.B. Tinker), Academic Press. London. 25-34.

Northcote, K.H. (1979). *A Factual Key for the Recognition of Australian Soils.* Rellim Technical Publications. Coffs Harbour, NSW.

O'Neill, E.G., O'Neill, R.V. and Norby, R.J. (1991). Hierarchy theory as a guide to mycorrhizal research on large scale problems. *Environmental Pollution,* **73**: 271-84.

Ocampo, J.A. (1980). Effect of crop rotations involving host and non-host plants on vesicular-arbuscular mycorrhizal infection of host plants. *Plant and Soil,* **56**: 283-91.

Ocampo, J.A. and Barea, J.M. (1985). Effect of carbamate herbicides on VA mycorrhizal infection and plant growth. *Plant and Soil,* **85**: 375-83.

Ocampo, J.A., Martin, J. and Hayman, D.S. (1980). Influence of plant interactions on vesicular-arbuscular mycorrhizal infections I. Host plant and non-host plants grown together. *New Phytologist,* **84**: 27-35.

Ockwell, A. (1990). The economic structure of Australian agriculture. In *Agriculture in the Australian Economy*, (Ed. D.B. Williams), Sydney University Press. Sydney. 27-49.

Office of the Commissioner for the Environment (1992). *1991 State of the Environment Report: Agriculture and Victoria's Environment*. Government of Victoria. Melbourne.

Olsen, J.K., Schaefer, J.T., Hunter, M.N., Edwards, D.G., Galea, V.J. and Muller, L.M. (1996). Response of capsicum (*Capsicum annuum* L.), sweet corn (*Zea mays* L.), and tomato (*Lycopersicon esculentum* Mill.) to inoculation with vesicular-arbuscular mycorrhizae. *Australian Journal of Agricultural Research,* **47**: 651-71.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA Circular No. 939. US Government Printer.

Parker, C.B. (1992). *The phosphorus balance of a conventional and bio-dynamic dairy farm*. Bachelor of Agricultural Science Thesis. La Trobe University, School of Agriculture.

Patterson, N.A., Chet, I. and Kapulik, Y. (1990). Effect of mycorrhizal inoculation on nodule initiation, activity, and contribution to legume productivity. *Symbiosis,* **8**: 9-20.

Pearson, C.J., Mann, I.G. and Zianhua, Z. (1991). Changes in root growth within successive wheat crops in a cropping cycle using minimum and conventional tillage. *Field Crops Research,* **28**: 117-33.

Penfold, C.M., Miyan, M.S., Reeves, T.G. and Grierson, I.T. (1995). Biological farming for sustainable agricultural production. *Australian Journal of Experimental Agriculture,* **35**: 849-56.

Peng, S., Eissenstat, D.M., Graham, J.H., Williams, K. and Hodge, N. (1993). Growth depression in mycorrhizal citrus at high-phosphorus supply. *Plant Physiology,* **101**: 1063-71.

Peoples, M.B., Lilley, D.M., Burnett, V.F., Ridley, A.M. and Garden, D.L. (1995). Effects of surface application of lime and superphosphate to acid soils on growth and N_2 fixation by subterranean clover in mixed pasture swards. *Soil Biology and Biochemistry,* **27**: 663-71.

Perry, M. and Hillman, B. (1991). *The Wheat Book- A Technical Manual for Wheat Producers*. Department of Agriculture, Western Australia, Bulletin No. 4196.

Pinkerton, A. and Randall, P.J. (1994). Internal phosphorus requirements of six legumes and two grasses. *Australian Journal of Experimental Agriculture,* **34**: 373-9.

Pirozynski, K.A. and Dalpé, Y. (1989). Geological history of the Glomaceae with particular reference to the mycorrhizal symbiosis. *Symbiosis,* **7**: 1-36.

Plenchette, C. and Perrin, R. (1992). Evaluation in the greenhouse of the effects of fungicides on the development of mycorrhiza on leek and wheat. *Mycorrhiza,* **1**: 59-62.

Plenchette, C., Fortin, J.A. and Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. *Plant and Soil,* **70**: 199-209.

Podolinsky, A. (1985). *Bio-dynamic Agriculture Introductory Lectures, Volume 1.* Gavemer. Sydney.

Podolinsky, A. (1989). *Bio-dynamic Agriculture Introductory Lectures, Volume 2.* Gavemer. Sydney.

Pope, P.E. and Holt, H.A. (1981). Paraquat influences development and efficiency of the mycorrhizal fungus *Glomus fasciculatus. Canadian Journal of Botany,* **59**: 518-21.

Porter, W.M., Robson, A.D. and Abbott, L.K. (1987a). Factors controlling the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *Journal of Applied Ecology,* **24**: 663-72.

Porter, W.M., Robson, A.D. and Abbott, L.K. (1987b). Field survey of the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *Journal of Applied Ecology,* **24**: 659-62.

Poulton, P.R. (1995). The importance of long-term trials in understanding sustainable farming systems: the Rothamsted experience. *Australian Journal of Experimental Agriculture,* **35**: 825-34.

Powell, C.L. and Daniel, J. (1978). Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertiliser from a phosphate-deficient soil. *New Phytologist,* **80**: 351-8.

Pratley, J.E. (1995). Long term investigations of the effect of tillage practices on crop production at Wagga Wagga, New South Wales. *Australian Journal of Experimental Agriculture,* **35**: 885-92.

Pringle, A. and Bever, J. (1996). Seasonal sporulation of eleven VAM species from an old-field in Durham, NC. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 98.

Puppi, G. and Bras, A. (1989). Nutrient and water relations of mycorrhizal white clover. *Agriculture, Ecosystems and Environment,* **29**: 317-22.

Raju, P.S., Clark, R.B., Ellis, J.R., Duncan, R.R. and Maranville, J.W. (1990). Benefit and cost analysis and phosphorus efficiency of VA mycorrhizal fungi colonisations with sorghum (*Sorghum bicolor*) genotypes grown at varied phosphorus levels. *Plant and Soil,* **124**: 199-204.

Rangeley, A., Daft, M.J. and Newbould, P. (1982). The inoculation of white clover with mycorrhizal fungi in unsterile hill soils. *New Phytologist,* **92**: 89-102.

Rayment, G.E. and Higginson, F.R. (1992). *Australian Laboratory Handbook of Soil and Water Chemical Methods*. Inkata Press. Melbourne.

Read, D.J. (1991). Mycorrhizas in Ecosystems. *Experimentia,* **47**: 376-91.

Reeves, T.G. (1976). Effect of annual ryegrass (*Lolium rigidum* Gaud.) on yield of wheat. *Weed Research,* **16**: 57-63.

Reganold, J.P. (1988). Comparison of soil properties as influenced by organic and conventional farming systems. *American Journal of Alternative Agriculture,* **3**: 144-55.

Reganold, J.P. (1995). Soil quality and profitability of biodynamic and conventional farming systems: a review. *American Journal of Alternative Agriculture,* **10**: 36-45.

Reganold, J.P., Palmer, A.S., Lockhart, J.C. and Macgregor, A.N. (1993). Soil quality and financial performance of biodynamic and conventional farms in New Zealand. *Science,* **260**: 344-9.

Reid, C.P.P. and Bowen, G.D. (1979). Effects of soil moisture on VA mycorrhiza formation and root development in *Medicago.* In *The Soil-Root Interface,* (Eds. J.L. Harley and S.R. Russel), Academic Press. London. 211-9.

Reid, R.L. (Ed.). (1990). *The Manual of Australian Agriculture.* Butterworths: Sydney.

Reuter, D.J. and Robinson, J.B. (1986). *Plant Analysis: An Interpretation Manual.* Inkata. Melbourne.

Rickert, K.G., Sedgley, R.H. and Stern, W.R. (1987). Environmental response of spring wheat in the south-western Australian cereal belt. *Australian Journal of Agricultural Research,* **38**: 655-70.

Riley, M.M., Adcock, K.G. and Bolland, M.D.A. (1993). A small increase in the concentration of phosphorus in the sown seed increased the early growth of wheat. *Journal of Plant Nutrition,* **16**: 851-64.

Römer, W. and Schilling, G. (1986). Phosphorus requirements of the wheat plant in various stages of its lifecycle. *Plant and Soil,* **91**: 221-9.

Ronsheim, M.L. and Sharma, K. (1996). The changing effects over time of mycorrhizae and phosphorus levels on the performance of *Allium vineale.* In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 102.

Rosendahl, C.N. and Rosendahl, S. (1990). The role of VAM in controlling damping-off and growth reduction in cucumber caused by *Pythium ultimum. Symbiosis,* **9**: 363-6.

Ryan, M.H. (1992). *Soil fungi on two adjacent wheat farms: comparative effects of organic and conventional management.* Honours thesis, Division of Botany and Zoology, Australian National University.

Ryan, M.H. (1997). The importance of the soil biota for the functioning of organic and biodynamic farms. In *Organic Agriculture in Australia. Proceedings of the national symposium on organic agriculture: research and development 30 June - 3 July 1996,* (Eds. D. Dumaresq, R. Greene and L. van Kerkhoff), RIRDC Research Paper No 97/14. Canberra. 116-34.

Ryan, M.H. and Ash, J.E. (1996). Colonisation of wheat in southern NSW by VA-mycorrhizal (VAM) fungi is significantly reduced by drought. *Australian Journal of Experimental Agriculture,* **36**: 563-9.

Ryan, M.H. and Dumaresq, D.H. (1994). Role of vesicular-arbuscular mycorrhizal fungi in SE Australian cereal cropping systems. In *Soil Biota: Management in Sustainable Farming Systems*, (Ed. C.E. Pankhurst). CSIRO. Adelaide. 147-50.

Ryan, M.H., Chilvers, G.A. and Dumaresq, D.C. (1994). Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. *Plant and Soil,* **160**: 33-40.

Sánchez-Díaz, M. and Honrubia, M. (1994). Water relations and alleviation of drought stress in mycorrhizal plants. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Systems*, (Eds. S. Gianinazzi and H. Schüepp), Birkhäuser Verlag. Basel. 167-78.

Sanders, I.R. and Fitter, A.H. (1992). The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species I Seasonal patterns of mycorrhizal occurrence and morphology. *New Phytologist,* **120**: 517-24.

Sanders, I.R. and Koide, R.T. (1994). Nutrient acquisition and community structure in co-occurring mycotrophic and non-mycotrophic old-field annuals. *Functional Ecology,* **8**: 77-84.

Sattelmacher, B., Reinhard, S. and Pomikalko, A. (1991). Differences in mycorrhizal colonisation of rye (*Secale cereale* L.) grown in conventional or organic (biological-dynamic) farming systems. *Journal of Agronomy and Crop Science,* **167**: 350-5.

Scheltema, M.A., Abbott, L.K. and Robson, A.D. (1987). Seasonal variation in the infectivity of VA mycorrhizal fungi in annual pastures in a mediterranean environment. *Australian Journal of Agricultural Research,* **38**: 707-15.

Schilthuis, W. (1994). *Biodynamic Agriculture*. Floris Books. Edinburgh.

Schreiner, R.P. and Koide, R.T. (1993). Mustards, mustard oils and mycorrhizas. *New Phytologist,* **123**: 107-13.

Schreiner, R.P., Mihara, K.L., Medaniel, H. and Bethlenfalvay, G.J. (1997). Mycorrhizal fungi influence plant and soil functions and interactions. *Plant and Soil,* **188**: 199-209.

Schweiger, P.F., Robson, A.D. and Barrow, N.J. (1995). Root hair length determines beneficial effect of a *Glomus* species on shoot growth of some pasture species. *New Phytologist,* **131**: 247-54.

Scott, E.P., Eason, W.R. and Scullion, J. (1996). Impact of agricultural management regimes on the effectivity of indigenous arbuscular mycorrhizal populations from soils under long-term pasture and grass-arable rotations in western UK. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 108.

Sen, R., Hepper, C.M., Azcon-Aguilar, C. and Rosendahl, S. (1989). Competition between introduced and indigenous mycorrhizal fungi (*Glomus* spp.) for root colonisation of leek. *Agriculture, Ecosystems and Environment,* **29**: 355-9.

Shafer, S. and Schoeneberger, M.M. (1994). Air pollution and ecosystem health: the mycorrhizal connection. In *Mycorrhizae and Plant Health*, (Eds. F.L. Pfleger and R.G. Linderman), APS Press. St. Paul. 153-88.

Shennan, C., Drinkwater, L.E., van Bruggen, A.H.C., Letourneau, D.K. and Workneh, F. (1991). Comparative study of organic and conventional tomato production systems: an approach to on-farm systems studies. In *Sustainable Agriculture Research and Education in the Field*, (Eds. B. Rice and J.P. Madden). National Academy Press. Washington DC. 109-33.

Short, K. (1994). *Quick Poison, Slow Poison: Pesticide Risk in the Lucky Country*, self published.

Simpson, D. and Daft, M.J. (1990). Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum. *Plant and Soil,* **121**: 179-86.

Sinnamon, L. (1996). Why grow food organically? *WellBeing Magazine,* **63**: 6-9.

Sivapalan, A., Morgan, W.C. and Franz, P.R. (1993). Monitoring populations of soil microorganisms during a conversion from a conventional to an organic system of vegetable growing. *Biological Agriculture and Horticulture,* **10**: 9-27.

Small, D., McDonald, J. and Wales, B. (1994a). *Alternative Farming Practices Applicable to the Dairy Industry*. Department of Agriculture, Victoria & The Dairy Research and Development Corporation. Kyabram.

Small, D.R. and McDonald, J. (1993). Biodynamic milk producing systems. In *Organic Agriculture- a serious farm of agriculture*, (Eds. D. Small, D. Auldist and J. Bourchier). The Australian Institute of Agricultural Science. Moama. 27-32.

Small, D.R., Wales, W. and McDonald, J.W. (1994b). Soils, pastures and milk production on bio-dynamic dairy farms in south-eastern Australia. In *10th International Organic Agriculture IFOAM Conference*, IFOAM. Lincoln University, New Zealand.

Smith, S.E. and Bowen, G.D. (1979). Soil temperature, mycorrhizal infection and nodulation of *Medicago truncata* and *Trifolium subterraneum. Soil Biology and Biochemistry,* **11**: 469-73.

Smith, S.E. and Gianinazzi-Pearson, V. (1990). Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: effects of photon irradiance and phosphate nutrition. *Australian Journal of Plant Physiology,* **17**: 177-88.

Smith, S.E. and Read, D.J. (1997). *Mycorrhizal Symbiosis*. Academic Press. San Diego.

Smith, T.F. (1978). A note of the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. *Australian Journal of Soil Research,* **16**: 359-61.

Smith, T.F. (1980). The effect of season and crop rotation on the abundance of spores of vesicular-arbuscular (V-A) mycorrhizal endophytes. *Plant and Soil,* **57**: 475-9.

Snellgrove, R.C., Splittstoesser, W.E., Stribley, D.P. and Tinker, P.B. (1982). The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. *New Phytologist,* **92**: 75-87.

Soderström, B.E. (1977). Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. *Soil Biology and Biochemistry,* **9**: 56-63.

Son, C.L. and Smith, S.E. (1988). Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. *New Phytologist,* **108**: 305-14.

Spokes, J.R., Hayman, D.S. and Kandasamy, D. (1989). The effects of funigicide-coated seeds on the establishment of VA mycorrhizal infection. *Annals of applied Biology,* **115**: 237-41.

Stace, H.C., Hubble, G.O., Brewer, R., Northcote, K.H. and Sleeman, J.R. (1968). *A Handbook of Australian Soils*. Rellim Technical Publications. Glenside, South Australia.

Steiner, R. (1993). *Spiritual Foundations for the Renewal of Agriculture- a course of lectures held at Koberwitz, Silesia, June 7 to June 16, 1924.* Bio-Dynamic Farming and Gardening Association Inc. Kimberton, Pennsylvania.

Stevens, T. (1997). Raiding the phosphorus bank. *Rural Research,* **174**: 13-6.

Stockdale, C.R. (1983). *The growth and utilisation of irrigated pastures grazed by dairy cows.* Master of Agricultural Science, Melbourne University.

Stribley, D.P., Tinker, P.B. and Rayner, J.H. (1980). Relation of internal phosphorus concentration and plant weight in plants infected by vesicular arbuscular mycorrhizas. *New Phytologist,* **86**: 261-6.

Stürmer, S.L. and Bellei, M.M. (1994). Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. *Canadian Journal of Botany,* **72**: 359-63.

Stutz, J.C. and Martin, C.A. (1996). Arbuscular mycorrhizal colonisation and sporulation in response to high temperatures and elevated atmospheric CO_2. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 113.

Sugavanam, V., Udaiyan, K. and Manian, S. (1994). Effect of fungicides on vesicular-arbuscular mycorrhizal infection and nodulation in groundnut (*Arachis hypogea* L.). *Agriculture, Ecosystems and Environment,* **48**: 285-93.

Sutton, P.J., Peterson, G.A. and Sander, D.H. (1983). Dry matter production in tops and roots of winter wheat as affected by phosphorus availability during various growth stages. *Agronomy Journal,* **75**: 657-63.

Sylvia, D.M. and Neal, L.H. (1990). Nitrogen affects the phosphorus response of VA mycorrhiza. *New Phytologist,* **115**: 303-10.

Sylvia, D.M., Hammond, L.C., Bennett, J.M., Haas, J.H. and Linda, S.B. (1993). Field response of maize to a VAM fungus and water management. *Agronomy Journal,* **85**: 193-8.

Tarafdar, J.C. and Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry,* **26**: 387-95.

Tarafdar, J.C. and Marschner, H. (1995). Dual inoculation with *Aspergillus fumigatus* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Na-phytate. *Plant and Soil,* **173**: 97-102.

Tennant, D. (1976). Root growth of wheat. I Early patterns of multiplication and extension of wheat roots including effects of levels of nitrogen, phosphorus and potassium. *Australian Journal of Agricultural Research,* **27**: 183-96.

Tester, M., Smith, S.E., Smith, F.A. and Walker, N.A. (1986). Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal infection and on the growth of infection units in *Trifolium subterraneum* L. *New Phytologist,* **103**: 375-90.

Thompson, J.P. (1987). Decline of vesicular-arbuscular mycorrhizas in long fallow disorder of field crops and its expression in phosphorus deficiency in sunflower. *Australian Journal of Agricultural Research,* **38**: 847-67.

Thompson, J.P. (1990). Soil sterilisation methods to show VA-mycorrhizal aid P and Zn nutrition of wheat in vertisols. *Soil Biology and Biochemistry,* **22**: 229-40.

Thompson, J.P. (1994a). Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. *Soil Biology and Biochemistry,* **26**: 1133-43.

Thompson, J.P. (1994b). What is the potential for management of mycorrhizas in agriculture? In *Management of Mycorhizas in Agriculture, Horticulture and Forestry,* (Eds. A.D. Robson, L.K. Abbott and N. Malajczuk), Kluwer. Netherlands. 191-200.

Thompson, J.P. and Wildermuth, G.B. (1989). Colonisation of crop and pasture species with vesicular-arbuscular mycorrhizal fungi and infection by *Bipolaris sorokiniana*. *Canadian Journal of Botany,* **67**: 687-93.

Thomson, B.D., Robson, A.D. and Abbott, L.K. (1986). Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytologist,* **103**: 751-65.

Thomson, B.D., Robson, A.D. and Abbott, L.K. (1992). The effect of long-term applications of phosphorus fertiliser on populations of vesicular-arbuscular mycorrhizal fungi in pastures. *Australian Journal of Agricultural Research,* **43**: 1131-42.

Tisdall, J.M. (1991). Fungal hyphae and structural stability of soil. *Australian Journal of Soil Research,* **29**: 729-43.

Tisdall, J.M. and Oades, J.M. (1979). Stabilization of soil aggregates by the root systems of ryegrass. *Australian Journal of Soil Research,* **17**: 429-41.

Tisdall, J.M. and Oades, J.M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science,* **33**: 141-63.

Tommerup, I.C. (1983). Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society,* **81**: 381-7.

Tommerup, I.C. (1984). Effect of soil water potential or spore germination by vesicular-arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society,* **83**: 193-202.

Tommerup, I.C. (1985). Inhibition of spore germination of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society,* **85**: 267-78.

Tommerup, I.C. and Briggs, G.G. (1981). Influence of agricultural chemicals on germination of vesicular-arbuscular mycorrhizas. *Transactions of the British Mycological Society,* **76**: 326-8.

Toro, M., Azcón, R. and Barea, J. (1997). Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. *Applied and Environmental Microbiology,* **63**: 4408-12.

Toth, R., Toth, D. and Starke, D. (1990). Vesicular-arbuscular mycorrhizal colonisation in *Zea mays* affected by breeding for resistance to fungal pathogens. *Canadian Journal of Botany,* **68**: 1039-44.

Trent, J.D., Svejcar, T.J. and Christiansen, S. (1989). Effects of fumigation on growth, photosynthesis, water relations, and mycorrhizal development of winter wheat in the field. *Canadian Journal of Plant Science,* **69**: 535-40.

Trent, J.D., Wallace, L.L., Svejcar, T.J. and Christiansen, S. (1988). Effect of grazing on growth, carbohydrate pools and mycorrhizae in winter wheat. *Canadian Journal of Plant Science,* **68**: 115-20.

van Bruggen, A.H.C. (1995). Plant disease severity in high-input compared to reduced-input and organic farming systems. *Plant Disease,* **79**: 976-84.

Vejsadová, H., Hrselová, H., Prikryl, Z. and Vancura, V. (1989). Effect of different phosphorus and nitrogen levels on development of VA mycorrhiza, rhizobial activity and soybean growth. *Agriculture, Ecosystems and Environment,* **29**: 429-34.

Verasan, V. and Phillips, R.E. (1978). Effects of soil water stress on growth and nutrient accumulation in corn. *Agronomy Journal,* **70**: 613-8.

Wallace, L.L. (1981). Growth, morphology and gas exchange of mycorrhizal and nonmycorrhizal *Panicum coloratum* L., a C_4 grass species, under different clipping and fertilization regimes. *Oecologia (Berlin),* **49**: 272-8.

Wallace, L.L. (1987). Mycorrhizas in grasslands: interactions of ungulates, fungi and drought. *New Phytologist,* **105**: 619-32.

Wander, M.M., Hedrick, D.S., Kaufman, D., Traina, S.J., Stinner, B.R., Kehrmeyer, S.R. and White, D.C. (1995). The functional significance of the microbial biomass in organic and conventionally managed soils. *Plant and Soil,* **170**: 87-97.

Wang, G.M., Stribley, D.P., Tinker, P.B. and Walker, C. (1985). Soil pH and vesicular-arbuscular mycorrhizas. In *Ecological Interactions in Soil,* (Ed. A.H. Fitter), Blackwell. Oxford. 219-24.

Warnock, A.J., Fitter, A.H. and Usher, M.B. (1982). The influence of a springtail *Folsomia candida* (insecta, collembola) on the mycorrhizal association of leek *Allium porrum* and the vesicular-arbuscular mycorrhizal endophyte *Glomus fasciculatus. New Phytologist,* **90**: 285-92.

Waters, J.R. and Borowicz, V.A. (1994). Effect of clipping, benomyl, and genet on ^{14}C transfer between mycorrhizal plants. *Oikos,* **71**: 246-52.

Watkinson, A.R., Newsham, K.K. and Fitter, A.H. (1996). The role of mutualisms in plant population dynamics. In *Frontiers of Population Ecology*, (Eds. R.B. Floyd, A.W. Sheppard and P.J. De Barro), CSIRO. Melbourne. 301-9.

Watson, D.M.H. and Millner, P.D. (1996). Assessment of Glomalean species biodiversity as influenced by trapping method. In abstracts from the *First International Conference on Mycorrhizae*, University of California, Berkeley. 125.

Watson, W.D., Reynolds, R.G., Collins, D.J. and Hunter, R.D. (1983). *Water 2000: Consultants Report No. 5: Agricultural Water Demand and Issues.* Australian Government Publishing Service. Canberra.

Webster, R. (1956). *Bygoo and Beyond.* Privately printed. Ardlethan.

Weir, R.G. and Cresswell, G.C. (1994). *Plant Nutrient Disorders 4: Pastures and Field Crops.* Inkata. Melbourne.

Werner, M.R. (1997). Soil quality characteristics during conversion to organic orchard management. *Applied Soil Ecology,* **5**: 151-67.

Werner, M.R. and Dindal, D.L. (1990). Effects of conversion to organic agricultural practices on soil biota. *American Journal of Alternative Agriculture,* **5**: 24-32.

Werner, M.R., Kluson, R.A. and Gliessman, S.R. (1990). Colonisation of strawberry roots by VA mycorrhizal fungi in agroecosystems under conventional and transitional organic management. *Biological Agriculture, and Horticulture,* **7**: 139-51.

Wetterauer, D.G. and Killorn, R.J. (1996). Fallow- and flooded-soil syndromes: effects on crop production. *Journal of Production Agriculture,* **9**: 39-41.

Whitelaw, M.A., Harden, T.J. and Bender, G.L. (1997). Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. nov. *Australian Journal of Soil Research,* **35**: 291-300.

Wilson, D.O. (1988a). Differential plant response to inoculation with two VA mycorrhizal fungi isolated from a low-pH soil. *Plant and Soil,* **110**: 69-75.

Wilson, J.B. (1988b). A review of evidence on the control of shoot:root ratio, in relation to models. *Annals of Botany,* **61**: 433-49.

Wilson, J.M. (1984). Competition for infection between vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* **97**: 427-35.

Wynen, E. (1988). *Sustainable and conventional agriculture in south-eastern Australia: a comparison*. La Trobe University, Discussion paper no. 22/88. Bundoora.

Wynen, E. (1992). *Conversion to organic agriculture in Australia: problems and possibilities in the cereal-livestock industry*. The National Association for Sustainable Agriculture Australia Ltd. Sydney.

Wynen, E. (1994a). Biodynamic and conventional dairy farming in Victoria: a financial comparison. In *Alternative Farming Practices Applicable to the Dairy Industry*, (Eds. D. Small, J. McDonald and B. Wales), Department of Agriculture, Victoria & The Dairy Research and Development Corporation. Kyabram. Appendix 6.

Wynen, E. (1994b). Economics of Organic Farming In Australia. In *The Economics of Organic Farming: An Economic Perspective*, (Eds. N.H. Lampkin and S. Padel), CAB International. Wallingford. 185-99.

Wynen, E. (1996). *Research Implications of a Paradigm Shift in Agriculture: The Case of Organic Farming*. Centre for Resource and Environmental Studies, Australian National University. Canberra.

Wynen, E. (1997). An economic assessment of organic agriculture and implications for future research. In *Organic Agriculture in Australia. Proceedings of the national symposium on organic agriculture: research and development 30 June - 3 July 1996*, (Eds. D. Dumaresq, R. Greene and L. van Kerkhoff), Rural Industries Research and Development Corporation. Canberra. RIRDC Research Paper 97/14, pp110-5.

Wynen, E. and Edwards, G. (1988). *Towards a Comparison of Conventional and Chemical-Free Farming in Australia*. School of Economics, La Trobe University. Bundoora.

Z.-Q., A., Guo, B.Z. and Hendrix, J.W. (1993). Populations of spores and propagules of mycorrhizal fungi in relation to the life cycles of tall fescue and tobacco. *Soil Biology and Biochemistry,* **25**: 813-7.

Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research,* **14**: 415-21.

Appendix One. Ardlethan Soil Profiles

Soil profile descriptions (Northcote 1979) from the organic farm at Ardlethan (Derrick 1996).

Profile 1: Red earth Gn 2.14

Horizon	Depth (mm)	Description
A1	0-140	Dark reddish brown (2.5 YR 3/4 moist, VC 5), whole coloured: silty loam; pH 6.5; crumb structure; smooth boundary to A2
A2	140-350	Dark reddish brown (5 YR 3/6 moist, VC 5), whole coloured; silty loam; pH 6.0; 50% crumb and 50% angular blocky structure; smooth boundary to B1
B1	350+	Dark reddish brown (5 YR 3/6 moist, VC 5), whole coloured; earthy fabric; silty clay; pH 6.0

Profile 2: Red earth Gn 2.12

Horizon	Depth (mm)	Description
A1	0-500	Dark reddish brown (5 YR 3/6 moist, VS 5), whole coloured; clay loam; pH 7.0; angular blocky structure; smooth boundary to B1
B1	500+	Reddish brown (5 YR 4/8 moist, VC 5), whole coloured; earthy fabric; fine sandy loam; pH 8.0

Appendix Two. Herbicides

Product name, active ingredients and target plants of herbicides used on conventional first year crops sampled on mixed farms in 1993, 1994 and 1995. For further information on weed control using herbicides in winter crops the reader is referred to Mullen and Dellow (1995).

Product name	Chemical name	Target plants
Avadex BW®	Tri-allate	wild oats
Hoegrass®	Diclofop methyl	grasses
Jaguar®	Bromoxynil + Diflufenican	broad-leaved weeds
Roundup®	Glyphosate 450 g L^{-1}	all plants
Tigrex®	MCPA + Diflufenican	broad-leaved weeds
Treflan®	Trifluralin	grasses
Tristar®	Diclofop + Fenoxaprop	grasses
2,4-D Ester 800	2,4-D Ester 800 g L^{-1}	broad-leaved weeds

Appendix Three. VAM Colonisation of Weeds

A limited examination was made of the VAM colonisation of the major weeds species found in the crops sampled at anthesis, 1993. The results give only a rough indication of VAM colonisation as sample sizes were often very small.

Species in the families Asteraceae, Fabaceae, and Poaceae were highly colonised, as is usually the case for species belonging to these three families (Gerdemann 1968). Species with low or no colonisation belonged to the families Polygonaceae and Fumariaceae, most members of which are considered to be non-mycorrhizal or rarely mycorrhizal (Gerdemann 1968).

There was no indication that the level of VAM colonisation influenced the farms on which the weeds were commonly found, that is, non-mycorrhizal weeds were not necessarily more common on the conventional farms, or *vice versa* (Appendix 4).

Family	Species	Common name	VAM (%)
Asteraceae	*Arctotheca calendula* (L.) Levyns	capeweed	49
	Chondrilla juncea L.	skeleton weed	59
	Silybum marianum (L.) Gaertner	variegated thistle	67
Fabaceae	*Trifolium* spp.	clover	41
Fumariaceae	*Fumaria* sp.	fumitory	0
Poaceae	*Avena fatua* L.	wild oats	58
	Lolium rigidum Gaud.	rye grass	33
Polygonaceae	*Emex australis* Steinh.	spiny emex	5
	Polygonum aviculare L.	wire weed	5

Appendix Four. Weed Abundance in 1993 Crops

The major species of weeds found in the first year wheat crops on each of the farms sampled during 1993. Occurrence was assessed visually after walking a transect across each paddock.

Farm	Species present		Occurrence
Yenda Conventional	*Avena fatua* (L.)	wild oats	occasional
	Lolium rigidum Gaud.	rye grass	occasional
	Chondrilla juncea L.	skeleton weed	rare
Yenda Organic	*Avena fatua* (L.)	wild oats	common
	Lolium rigidum Gaud.	rye grass	common
	Chondrilla juncea L.	skeleton weed	occasional
	Arctotheca calendula (L.) Levyns	capeweed	rare
Ardlethan Conventional	*Avena fatua* (L.)	wild oats	rare
	Lolium rigidum Gaud.	rye grass	rare
	Polygonum aviculare (L.)	wireweed	rare
Ardlethan Conversion	*Avena fatua* (L.)	wild oats	common
	Polygonum aviculare (L.)	wireweed	common
	Chondrilla juncea L.	skeleton weed	occasional
	Lolium rigidum Gaud.	rye grass	occasional
	Emex australis Steinh.	spiny emex	rare
Ardlethan Organic	*Lolium rigidum* Gaud.	rye grass	common
	Avena fatua (L.)	wild oats	occasional
	Emex australis Steinh.	spiny emex	occasional
	Arctotheca calendula (L.) Levyns	capeweed	rare
	Echium plantagineum (L.)	Paterson's curse	rare
	Trifolium spp.*	clovers	rare
Cootamundra Biodynamic	*Lolium rigidum* Gaud.*	rye grass	common
	Trifolium spp*.	clovers	common
	Papaver hybridum L.	poppy	occasional
	Avena fatua (L.)	wild oats	rare
	Fumaria sp.	fumitory	rare
	Silybum marianum (L.) Gaertner	variegated thistle	rare

* Deliberately undersown to aid in pasture establishment

Appendix Five. Information on Individual Dairy Farms

Selected information on individual dairy farms sampled in NE-Victoria; years since last tillage, time since P fertiliser last applied, the rate and fertiliser type of the last P fertiliser on the conventional farms and the years since the biodynamic preparation BD500 was added on the biodynamic farms. Data are based on farmer estimates for the paddock on each farm sampled as part of this project in March 1993. Note that time since last fertiliser addition is given in months for the conventional farms and in years for the biodynamic farms. Fertilisers applied were single superphosphate (SSP), superphosphate (SP), diammonium phosphate (DAP) and rock phosphate (RP).

Pair	Conventional				Biodynamic		
	Tilled (y.a.)	P fertiliser (m.a.)	-type	-rate (kg ha^{-1})	Tilled (y.a.)	P fertiliser (y.a.)	BD 500 (y.a.)
1	25	12	SP	370	40	2 (RP)	1
2	30	5	SSP	200	30	20	1
3	na	5	SSP	200	30	> 30	0.5
4	> 8	0.5	DAP	120	> 30	> 10	1
5	20	12	SP	500	> 30	18	1
6	25	4	SP	250	40	> 10	0.5
7	> 30	6	SP	300	> 30	> 30	0.5
8	> 20	28	SP	500	> 20	10	1
9	25	6	SSP	300	30	20	0.5
10	20	6	SSP	410	8 *	4	0.5

* laser levelled

Appendix Six. The Importance of the Soil Biota for the Functioning of Organic and Biodynamic Farms

Reprint of; Ryan, M.H. (1997). The importance of the soil biota for the functioning of organic and biodynamic farms. In *Organic Agriculture in Australia. Proceedings of the national symposium on organic agriculture: research and development 30 June - 3 July 1996*, (Eds. D. Dumaresq, R. Greene and L. van Kerkhoff), RIRDC Research Paper No 97/14. Canberra. 116-34.

1. Introduction

Organic and biodynamic (alternative) farmers both claim that enhancing the functioning of the soil biota is critical to the successful functioning of their farms: a belief echoed by researchers and reviewers (Lopez-Real, 1985; La Rooj, 1989; National Research Council, 1989; Wynen, 1992; Macgregor, 1994; Penfold *et al.*, 1995; Sinnamon, 1996). Indeed the Australian National Standard for Organic and Biodynamic Produce states that such produce is defined by being "produced *in soils of enhanced biological activity, determined by the humus level, crumb structure and feeder root development, such that plants are fed through the soil ecosystem and not primarily through soluble fertilisers added to the soil*" (A.Q.I.S., 1992).

This statement suggests that a particularly important role of soil organisms is to supply plants with nutrients from relatively insoluble sources in the soil. It also implies that soil biological activity is either reduced, or of little significance, on conventional farms.

This paper begins with a broad summary of the role of the soil biota and then reviews the existing evidence regarding the role of soil organisms in the functioning of alternative systems, generally by referring to studies where comparisons have been made between farms under alternative management and farms under conventional management. Soil organisms are examined under four broad headings: microbial biomass/activity; soil fauna; pathogens; and symbiotic organisms involved in plant nutrient uptake. Australian examples are used when possible, but as limited literature is available, overseas studies are also referred to when necessary. Conclusions are drawn about whether the composition and functioning of the soil biota will differ between alternative and conventional farms under Australian conditions and the reasons for any such differences.

2. The Soil Component of the Ecosystem

The functioning of the soil biota is likely to influence the functioning of the entire ecosystem (Price, 1988). Most organisms in terrestrial ecosystems live in the soil and

the biomass and production of the soil organisms is often far greater than that of the above-ground organisms. For instance, Coleman *et al.* (1976) estimated that below ground production in a lightly grazed prairie was 83% of total production. Also, soil microorganism communities are extremely diverse, often with large populations and short generation times and consequently their capacity to respond to environmental change is generally much greater than that of organisms at higher trophic levels.

In addition, some of the key links in terrestrial ecosystems are in the soil and consequently the soil ecosystem effects the functioning of organisms at all trophic levels. For instance, plant growth is mediated by the availability of nutrients and water in the soil. Nutrient cycling primarily occurs through biological decay processes in the soil which transform nutrients from organic forms back to ionic forms that plants can access. Soil organisms may also significantly influence plant growth by directly providing nutrients, particularly nitrogen (N) and phosphorus (P), to plants through symbiotic relationships. Soil organisms also contribute to soil structure through their role in the formation and stabilisation of soil aggregates.

Thus soil organisms are involved in soil processes which are vital for the healthy functioning of agricultural systems. A diverse and complex soil biota may also contain mechanisms to maintain equilibrium and may contribute towards a agricultural system being relatively resilient to change and unlikely to suffer outbreaks of pathogens.

However, until recently, the soil biota has received relatively little attention from agricultural researchers. In part, this may be due to many of the major functions of soil organisms being considered redundant in systems where the inputs and management techniques of modern industrialised agriculture are used. For instance, the role of soil organisms in the release of nutrients in a form available to plants may be less critical when large quantities of soluble fertiliser are being added to a system. In addition, there are inherent difficulties in researching and developing an understanding of complex systems such as the soil ecosystem.

The abundance and activities of soil organisms are both controlled by environmental constraints, in particular, the level of soil moisture, soil temperature and the availability of an energy source, generally organic matter. All farming practices, especially crop rotations, tillage, stubble management, fertiliser application, biocide application and irrigation, will affect the soil biota through their influence on these environmental factors. Any consistent differences in the soil biota between alternative and conventional agricultural systems should arise from differences in management practices. Studies which have examined the soil organisms present on alternative and conventional farms are reviewed below.

3. Comparisons of Soil Biota between Alternative and Conventional Farms

a) Microbial biomass / activity & the microflora (bacteria and fungi)

The microflora generally constitute the largest portion of the biomass of organisms in the soil. They are involved in many soil processes and are particularly important in cycling of nutrients. However, the large volumes and number of individual species of bacteria and fungi in the soil makes quantification of the biomass and activities of individual species virtually impossible. Consequently, researchers often make broad measures of these organisms through methods which estimate microbial biomass or activity. Although there are some cases, discussed later, where particular microbial groups have a prominent function which makes measurement possible, for instance, pathogens and symbionts which directly affect plant growth.

Total microbial biomass is generally broadly positively correlated with the level of soil organic matter (carbon) (Witter *et al.*, 1993). For example, in a long term field trial in Sweden, Schnürer *et al.* (1985) examined a number of treatments involving cropping of wheat with treatments of artificial fertilisers, straw incorporation, and farmyard manure. Both microbial biomass estimates and activity measurements showed a highly significant correlation with soil organic matter levels.

It appears that additions of large volumes of organic matter will generally lead to increased microbial biomass. For instance, in an Australian trial comparing growth of vegetables under organic and conventional management, the populations of fungi, bacteria and actinomycetes were found to be greater in the organic plots where a high rate of compost (80-120 t ha^{-1}) had been added (Sivapalan *et al.*, 1993).

Studies conducted overseas on alternative and conventional farms have tended to produce similar results to Sivapalan *et al.* (1993). Reganold (1988) examined a pair of adjacent organic and conventional farms in Washington State, USA. The organic farm had higher soil enzyme levels and microbial biomass than the conventional farm, indicating a more active microbial community. Differences were attributed to the inclusion of a green manure crop every third year in the crop rotation of the organic farm and different tillage practices. Elmholt and Kjøller (1989) also found higher numbers of saprophytic fungi in soil from a Danish biodynamic farm compared with a conventional farm. The results were attributed to the biodynamic farm including legumes and grasses in the rotation, regularly applying composted manure and not applying pesticides.

Often when alternative farms have been found to have higher microbial biomass, there has been the inclusion of legumes in the rotation. Robertson and Morgan (1996) found that cropping with legumes had a positive effect on soil fungi, microbial biomass and soil water content . It was suggested that this could have been due to improved water retention by decomposition and humification of the residues, as much to increased carbon and N supply (Robertson and Morgan, 1996).

However, many other management practices and environmental parameters may influence soil organic matter levels and microbial biomass. For instance, Sivapalan *et al.* (1993) also found that plots which were previously under pasture for 10 years supported a higher microbial population than those previously cropped with vegetables: an effect which continued for up to three years after the pasture was removed. Lower soil organic matter, greater soil disturbance and greater use of pesticides on the previously cropped plots may all have contributed to this result. Robertson and Morgan (1996) also found that compared with pastures, cultivated and harvested crops generally produced less organic residues, partly because soil carbon and N reserves were reduced by cultivation. Reducing tillage has been shown to result in increases in soil microorganisms (Gupta and Roper, 1994). Sivapalan *et al.* (1993) also noted that a halving in the soil moisture content at one sampling date resulted in low numbers in all microbial groups.

However, addition of organic matter does not necessarily result in increased levels of soil microorganisms. Robertson and Morgan (1996) studied the effects of converting vegetable plots to organic management at Frankston, Victoria. They found that 2-4 fold increases in annual organic matter inputs, applied as compost (6 - 42 t ha^{-1} supplying 50 - 352 kg ha^{-1} N), did not increase microbial biomass. They attributed this to either the quantity or quality of the compost, other factors limiting soil microbes such as water, or sudden bursts in activity which were not detected by their sampling. They also noted that while the labile fractions of organic matter additions will be responsible for short-term fluctuation in microbial biomass, older stabilised residues are probably more important in determining the long-term size of the biomass. Support for this conclusion comes from a long term experiment in Sweden where Witter *et al.* (1993) found the soil microbial biomass to be predominantly influenced by soil organic matter with an age of 30 years or more. It was also noted that organic matter added to the soil was no longer available, or was insignificant in amount relative to the older material, within one year (Witter *et al.*, 1993).

Thus, even if regular organic matter additions are greatly increased after a farm converts to alternative management, it may be a long time before these are reflected in

soil organic carbon levels. For instance, Penfold *et al.* (1995) examined South Australian trials which compared organic, biodynamic, integrated, and conventional broadacre farming systems in large replicated field plots. It was found that 6 six years of radically different management had no significant effect on organic carbon levels. However, there was an indication that organic and biodynamic treatment plots had higher levels of microbial activity, as measured by loss of tensile strength by cotton strips. Similarly, Derrick (1996) found that after 30 years of stubble retention and organic management, soil organic matter levels were 1.17 mg kg^{-1} on an organic wheat property in south-east Australia and 1.06 mg kg^{-1} on a conventional neighbour where stubble was routinely burnt: a non-significant difference.

In addition, the extensive nature of most agriculture in Australia means that acquiring, composting and spreading large volumes of organic matter is simply not practicable. Consequently, the volume of organic matter applied on most broadacre Australian alternative farms would not differ from their conventional neighbours, particularly when stubble retention, which is increasingly popular with conventional farmers, is practiced by all farmers. Thus any differences in microbial biomass between alternative and conventional farms would generally have to result from the other factors such as tillage or rotation. However these may also differ only a little between many alternative and conventional farms.

For instance, in the most comprehensive comparison of alternative and conventional farms in Australia, 10 pairs of biodynamic and conventional irrigated dairy farms in Victoria were examined by Small *et al.* (1994). The biodynamic farms had been managed biodynamically for, on average, 16 years. In paddocks under permanent pasture, organic carbon levels and soil microbial biomass did not differ between the two management systems (Small *et al.*, 1994).

The lack of differences in the above study are of additional interest as all the biodynamic farms were regularly applying the homeopathic preparation BD500. BD500 is applied for a variety of reasons, including to stimulate soil life. Penfold *et al.* (1995) also found BD500 to have no detectable impact on the microbial biomass over two years of measurement. However, it is possible that the preparation affects the biomass and activity of particular organisms: an effect which may not be detected by broad measures of biomass or activity.

Ryan (1992) examined cellulose decomposing fungi in soil from neighbouring organic and conventional wheat farms: the organic farm had been retaining stubble for 30 years and the conventional farmer generally burnt crop stubble. Over 40 species of fungi were distinguished by their colony morphology on cellophane which had been placed

on slides in the soil. Overall, species diversity appeared similar on the two farms, although there were a number of species that were present only on one farm, or were more numerous on one of the farms. There was an indication that cellulose decomposing ability was higher on the organic farm, but the results were not significant. As mentioned above, organic carbon levels were higher on the organic farm, but the difference was not statistically significant (Derrick, 1996). In other studies, retention of crop stubble has been shown to result in increased population sizes of cellulolytic bacteria and fungi (Gupta and Roper, 1994).

Thus there is some indication that alternative farms tend to have higher levels of microbial biomass, particularly if their management regime includes addition of high levels of organic matter, such as compost. Inclusion of pasture phases and legumes in the rotation and stubble retention will also tend to increase microbial biomass. If soil organic carbon levels are similar, then microbial biomass may not differ between alternative and conventional farms. However, even under these circumstances, differences in the biomass or activities of specific groups of microorganisms may still exist between alternative and conventional farms. This is an area which has received little attention from researchers.

b) Soil fauna

Like the microflora, soil micro-, meso-, and macrofauna are responsible for the decomposition of organic matter in the soil and, due to their larger size, also tend to play a role in the movement of organic matter through the soil profile and its initial fragmentation. Being larger and less numerous than the microflora, the investigation of the activities of individual genera and species has been possible and current knowledge for agricultural situations has been summarised by Gupta (1994) and Fraser (1994).

One group of macrofauna which has received a large amount of attention are the earthworms. Worms can have dramatic positive effects on soil structure and fertility and there is a lot of interest in enhancing their roles in agricultural systems (Baker *et al.*, 1994; Buckerfield and Auhl, 1994). In an Australian study of biodynamic and conventional irrigated dairy farms, Small *et al.* (1994) found a higher biomass of worms on the conventional farms (87 g m^{-2}) in comparison to the biodynamic farms (59 g m^{-2}). Two factors were considered to be responsible for this result. First, on the biodynamic farms, milk production was limited by P. The resulting lower milk production required less intake of feed, leading to a lower faecal output on the biodynamic farms and consequently less food available for worms. Secondly, the longer irrigation intervals over summer on the biodynamic farms may have reduced worm numbers.

Springett (1994) examined two pairs of adjacent organic and conventional farms in New Zealand, one dairying and the other mixed cropping. Indices of species diversity, richness, and evenness for soil fauna were slightly higher for the organic farms. Two key species, oribatid mites and spiders, were present on the organic farms and absent from the conventional farms. Earthworm numbers were greater on the organic farms, however species composition was the same. On the dairy farms the length of earthworm burrows was much higher on the organic farm (410 cm m^{-2}) than on the conventional (154 cm m^{-2}) (Springett and Gray, 1994). It was not determined which management practices were responsible for these differences.

Werner and Dindal (1990) examined the soil biota in the 5th year of a trial in Pennsylvania where three treatments had been established: organic-manure, organic-legume, and conventional. They found that several groups of organisms were more abundant and more active during various portions of the growing season in the organic treatments. Nematodes, prostigmatid mites and collembola were all more abundant on the organic plots, apparently in response to the organic matter inputs. The predatory mesostigmatid mites were also most abundant in organic-manure plots and appeared to be positively affected by weed growth in the plots. Oribatid mites were strongly suppressed in all treatments by tillage, and earthworms were also strongly negatively affected by tillage.

Thus, as with the microflora, the level of organic matter in the soil is probably the most important factor in regulating soil fauna populations, as it directly or indirectly provides all organisms with food and energy. Additions of organic matter may quickly result in increased numbers of organisms such as earthworms (Werner and Dindal, 1990; Penfold *et al.*, 1995) and management practices which reduce organic matter levels, such as tillage and stubble removal, may quickly have a negative effect on population levels (Werner and Dindal, 1990; Pankhurst *et al.*, 1995; Penfold *et al.*, 1995). Soil fauna are also likely to be significantly affected by environmental factors such as soil moisture and other management factors such as rotation, and fertiliser and pesticide application.

c) Pathogens

Management practices can significantly effect the occurrence of soil-borne diseases and may be used to reduce outbreaks to acceptable levels. However few management strategies give consistent responses across a range of environments and the majority are unpredictable, partly due to a lack of understanding of the mechanisms involved (Neate, 1994).

The literature on severity of plant disease on alternatively managed farms has been reviewed by van Bruggen (1995) who found that root diseases and pests generally occurred at similar levels on alternative and conventional farms or were less severe on alternative farms. Foliar diseases showed more variability, perhaps due to the large influence of climatic factors on these diseases, although some foliar diseases have been found to increase when N fertiliser is used (Daamen *et al.*, 1989). The lower levels of root disease and severity on some alternative farms was ascribed to the regular application of organic matter and elimination of pesticides. Together these were assumed to have increased the general level of soil microbial activity and have resulted in increased competition and/or antagonism in the soil. The lower disease level on the alternative farms was also thought to have been influenced by longer rotations breaking the disease cycle (van Bruggen, 1995).

Workneh and van Bruggen (1994) examined the soil organisms present in the rhizosphere of tomato plants grown on organic and conventional farms in California. The most significant differences between the samples were the higher populations and increased diversity of actinomycetes in the organic soil. As actinomycetes play an important role in organic matter decomposition, this difference was ascribed to the use of compost and green manures on the organic farms. Overall, the soils from the organic farms had higher microbial activity and the level of microbial activity correlated strongly with suppression in severity of the disease corky root (*Pyrenochaeta lycopersici*).

Similarly, in an Australian trial comparing the growth of vegetables under organic and conventional management, a number of soil-borne pathogens including *Alternaria brassicicola*, *Botrytis cinerea*, and *Rhizoctonia solani*, were found only on the conventional plots (Sivapalan *et al.*, 1993). Antagonistic fungi, fungi with the potential to kill or weaken pathogens or prevent their infection, were found in much greater frequencies in soil from the organic plots. It was suggested that the addition of organic matter (80- 20 t ha^{-1} of composted manure, brown coal and grass clippings) to the organically farmed plots had encouraged the presence of antagonistic fungi. Thus when organic management involves addition of large amounts of compost it is possible that the levels of antagonistic organisms in the soil will increase and reduce the incidence of disease (Hoitink and Fahy, 1986).

There have been no quantitative studies of the levels of soil-borne diseases on broadacre alternative farms in Australia, systems where large inputs of compost are unlikely to occur. Although, anecdotal evidence suggests that after a 3-4 year conversion period, farms converted from conventional to alternative management will

then experience lower levels of disease than before conversion. Researchers of conventional systems have found that, under certain circumstances, over time soils may simply become suppressive for a particular disease. For instance, in a long-running field trial, Roget (1995) found that the incidence of rhizoctonia root rot (*Rhizoctonia solani*) increased to severe levels over the initial 5 years and then decreased to negligible levels over the following 6 years. This phenomena has been noted elsewhere and is presumed to result from a build-up of populations of suppressive soil organisms. However, the mechanisms behind suppressive soils are not fully understood and it is generally not possible to predict their development. It is possible that suppressiveness may build-up on alternative farms after conversion, thus the initial increase in disease incidence noted by Roget (1995), could correspond to the conversion period when yields have been found to decrease (Janke *et al.*, 1991; Wynen, 1992).

d) Symbioses enhancing plant nutrient uptake

In view of the nutrient poor status of many Australian soils (Leeper and Uren, 1993), a group of potentially important soil organisms are those which form symbiotic relationships with host plants and result in plant nutrient uptake being enhanced. As alternative farmers tend to apply less fertiliser and/or use relatively insoluble fertilisers, it is possible that these relationships are of particular importance in alternative systems. Two organisms which form symbiotic relationships with many agricultural plant species are bacteria in the genus *Rhizobia* and vesicular-arbuscular mycorrhizal fungi.

Rhizobium form a relationship with legumes and have been widely utilised in Australian agriculture. As these bacteria fix atmospheric N and supply it to the host plant, they are likely to be an important part of any sustainable agricultural system. Proper management of legume crops and pastures can eliminate the necessity to add N fertiliser. For instance, agriculture in south-east Australia is largely based on rotation of cereals with pasture containing legumes. Five to eight years of pasture containing subterranean clover can provide sufficient N to support up to three subsequent cereal crops (A. Ellington, unpublished data in (Coventry *et al.*, 1985). In these systems, soil pH is an important factor controlling nodulation, with liming of acid soils improving nodulation (Coventry *et al.*, 1985).

In a comparison of irrigated pasture on biodynamic and conventional dairy farms, the number of nodules on white clover was found to correlate positively with the concentration of P in the shoots of pasture plants (Figure 1; M. Ryan, unpublished data). Consequently, the level of nodulation tended to be lower on biodynamic farms where P fertiliser was not applied. This effect was probably mediated through the host plant (Ledgard and Steele, 1992), with clover in conventional pasture having enhanced

growth due to better P nutrition and therefore being able to support more nodules. Lower nodule numbers may, therefore, be reducing N-fixation and further limiting pasture growth on the biodynamic farms.

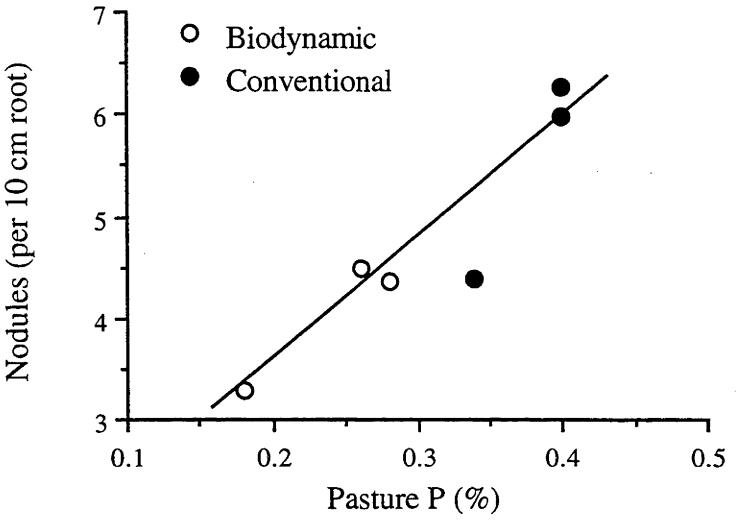


Figure 1. Relationship between frequency of nodules on roots and pasture P concentration in permanent pasture on 3 pairs of biodynamic and conventional dairy farms in northern Victoria (mean of 15 sites per paddock).

A second widespread group of symbiotic organisms are vesicular-arbuscular mycorrhizal (VAM) fungi. Mycorrhizal fungi are obligate symbionts, relying on the host plant for all their carbon requirements. In return, the fungi provide the plant with nutrients, particularly P. VAM fungi colonise the roots of most crop and pasture species, with canola and lupins being two of the few exceptions. The level of dependency of plant species varies, but a low level of colonisation can result in significantly reduced plant growth in dependent species such as linseed (Thompson, 1994).

Alternative farms have often been found to have higher levels of VAM colonisation than conventional neighbours (Lengnick and King, 1986; Bokhorst, 1989; Werner *et al.*, 1990; Sattelmacher *et al.*, 1991; Ryan *et al.*, 1994). In a 4 year study of VAM on wheat properties in south-east Australia, (Ryan *et al.*, 1994; Ryan, unpublished data) crops on organic and biodynamic farms consistently had significantly higher levels of VAM colonisation than neighbouring conventional farms. Results from the 1993 season are shown in Figure 2: the differences were most marked in the early stages of growth prior to tillering, 75 days after sowing.

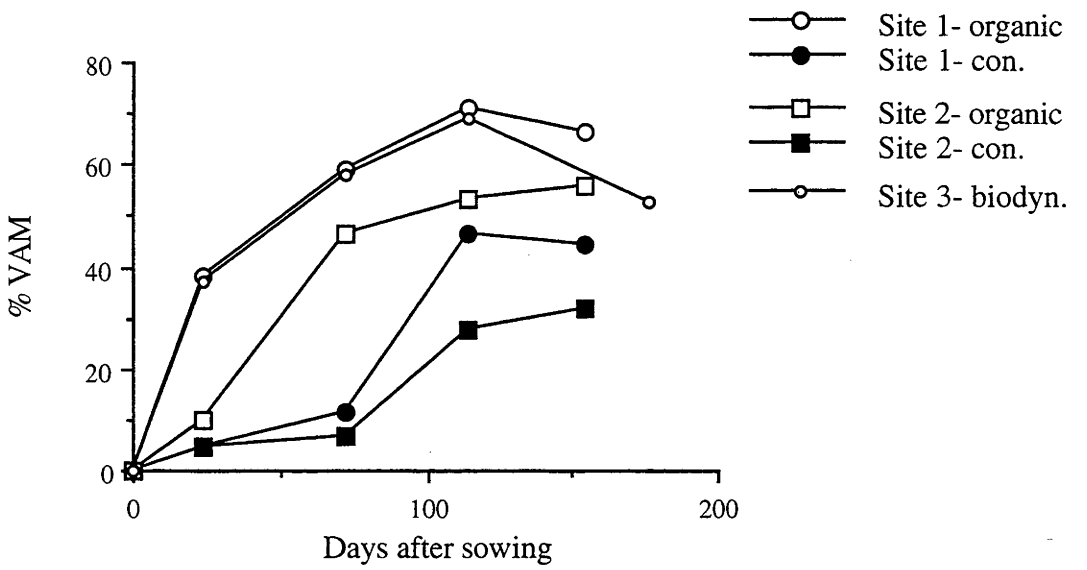


Figure 2. Percentage of root length colonised by VAM fungi in paired wheat crops at three sites in SE-Australia over the 1993 wheat season (mean of 20 sites per paddock).

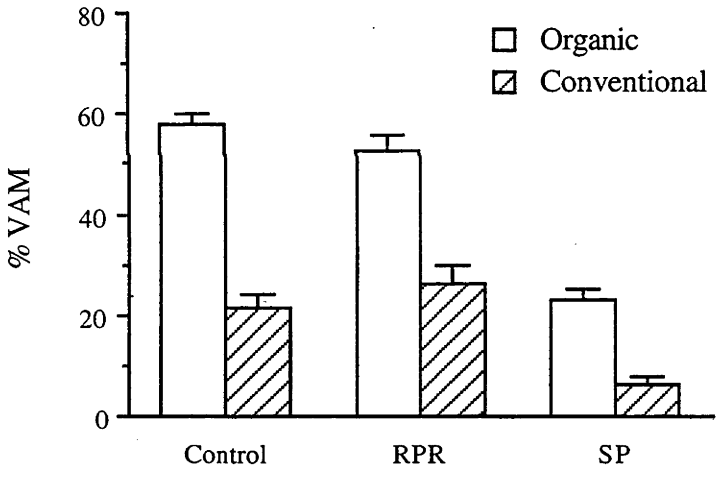


Figure 3. Percentage of wheat root length colonised by VAM fungi at tillering in fertiliser trials on adjacent organic and conventional farms. Treatments applied were no fertiliser (control) and 40 kg ha^{-1} of P as reactive rock phosphate (RPR) or superphosphate (SP) (mean ± s.e.m, 20 sites per treatment).

In fertiliser trials conducted on one pair of farms it was again found that wheat on the organic farm had higher levels of VAM colonisation (Figure 3). On each farm the addition of superphosphate significantly reduced the level of VAM colonisation, while the less soluble reactive rock phosphate had no effect (Dann *et al.*, 1996). Thus the lower levels of VAM colonisation in conventional crops compared with organic neighbours, shown in Figure 2, are due to use of superphosphate on the conventional farms. When the host-plant has easy access to available P it has no need for the fungi

and consequently when the concentration of P in the host plant is high, VAM colonisation is restricted.

VAM fungi may influence other aspects of the soil ecosystem, including reducing the effects of pathogenic organisms (Thompson and Wildermuth, 1989), enhancing plant uptake of other micronutrients such as zinc (Thompson, 1994) and improving soil structure (Tisdall and Oades, 1979). Factors which may influence the level of VAM in Australian systems are summarised by Abbott and Robson (1994). In addition to soluble P fertilisers, VAM colonisation levels may be substantially reduced by long fallows (Thompson, 1994) and drought (Ryan and Ash, 1996).

Thus, for two soil organisms known to enhance plant uptake of nutrients, the evidence is contradictory. Biological fixation of N by Rhizobia may be limited by lack of P on alternative farms, while low P availability may enhance levels of VAM colonisation. However, as shown in section 4, the enhanced levels of VAM on the organic farm do not necessarily compensate for the effects of lower levels of soil available P on crop growth.

4. Case Study: Adaptation of the Soil Biota to 30 years of Organic Management

The complexity of the soil ecosystem often makes it difficult to assess the contribution of individual components to the functioning of the entire system. Thus even if a difference is identified between alternative and conventional systems, it may still not be possible to draw conclusions about the effect of this on the functioning of each system. Under such circumstances it may be more useful to measure the functioning of the entire system. This was attempted by Dann *et al.* (1996) who examined growth of wheat under various rates and types of fertiliser addition on adjacent conventional and organic farms.

The farms were located in the south-east wheatbelt of NSW where P was the major limiting factor for crop growth and yield. The organic farm had ceased applying superphosphate 30 years previously and had subsequently been applying rock phosphate to crops. Rock phosphate contains P in a relatively insoluble form, however, it was speculated that over 30 years the soil biota may have adjusted in response to the use of rock phosphate, and perhaps the organic management regime in general, to allow plants to access the P from the fertiliser. Conversely, it was thought that on the conventional farm where the plants had been supplied with soluble P from superphosphate for many years, the biological mechanisms to allow plants to access the P in rock phosphate may no longer be present, or may have been less efficient than on the organic farm.

To test these hypotheses, yield of wheat grown under various rates of superphosphate and rock phosphate was compared with an unfertilised control on each farm in two consecutive years. In 1991, a relatively dry year, there was little difference in yield between the trials on each farm (Dann *et al.*, 1996). However, in 1992, a wetter year, yields were much higher on the conventional farm (Figure 4). In both years the addition of superphosphate increased crop growth and yield on both farms, while the addition of rock phosphate had no significant effect. Thus after 30 years of organic management and rock phosphate addition, there was no evidence that the soil biota on the organic farm was better able to make the P in the rock phosphate available to the crop than the conventional soil biota. Indeed the similar growth and yield of the crop in the control and the rock phosphate treatments on both farms indicates that the wheat plants were not able to access significant amounts of P from the rock phosphate, even when heavily colonised by VAM fungi.

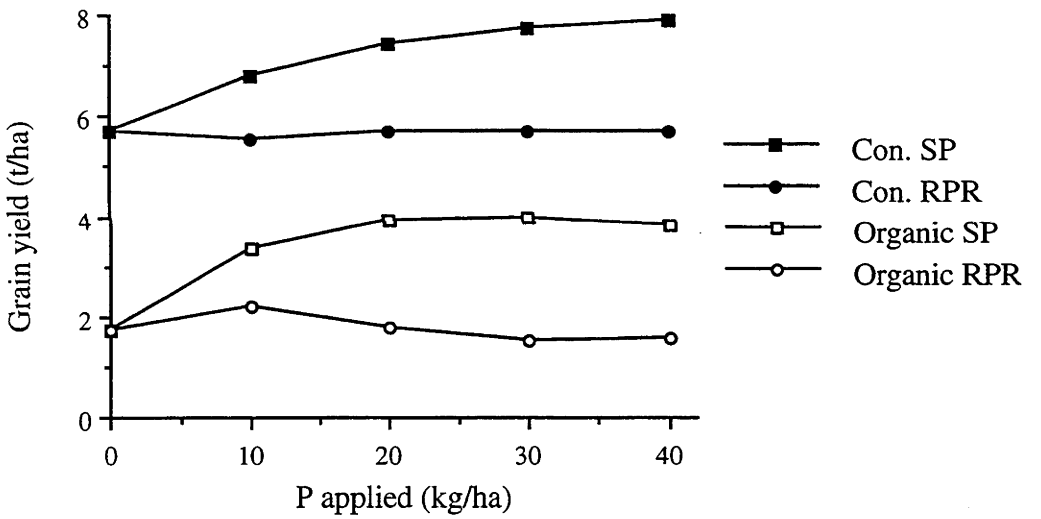


Figure 4. Response of wheat to addition of 4 rates of superphosphate (SP) and reactive rock phosphate (RPR) in field trials conducted on adjacent organic and conventional farms (Dann *et al.*, 1996).

Thus the often repeated claim that on organic farms the soil life "releases substances that transform insoluble natural fertilisers into readily available plant nutrients" (Sinnamon, 1996), was not found to be the case, even thought the nutrient involved, P, was the major limiting nutrient for plant growth. However, it is possible that while the P from the rock phosphate wasn't being utilised, uptake of P from other sources in the soil was being stimulated by soil organisms, and may have been significantly contributing to growth of the control and rock phosphate treatments on one or both farms. The environment at the trial site is not considered favourable for the effective use of rock phosphate (Bolan *et al.*, 1990), and it is possible that different results would

be obtained under conditions of higher rainfall.

5. Summary: Factors Responsible for Influencing Soil Biological Activity on Alternative Farms

It appears that the level of soil microbial activity may vary between alternative and conventional farms, with alternative farms having higher levels under some circumstances. The differences appear to occur in response to specific management practices, discussed briefly below. The most significant factor influencing soil biological activity appears to be soil carbon levels and additions of organic matter.

Addition of large quantities of organic matter, such as compost, is often a feature of alternative systems of agriculture in Europe. However, in Australia, the composting and spreading of organic materials is often not such an integral part of our agricultural systems, due to their extensive nature. In addition, the concentration of population along the eastern seaboard makes the transport and use of urban waste in many major agricultural areas prohibitively expensive. However, intensive horticultural operations or orchards may potentially be able to produce and apply large volumes of organic matter. Soil organic matter levels can also be increased through use of green manure crops, longer pasture phases, and retaining stubble. Although not exclusive to alternative farming systems, these are all practices which are often employed by alternative farmers. Further research is required to quantify to effect of these management practices. In particular, research into the role of organic matter additions, especially compost, in stimulation of antagonistic organisms and development of suppressive soils could prove profitable.

A management practice which is likely to have a large negative impact on the soil biota is tillage. Tillage acts both through its negative effect on soil organic matter levels and also through its effects on organisms not adapted to cope with disturbance (Werner and Dindal, 1990). The combination of reduced tillage and stubble retention has been found to increase soil microflora and microfauna (Gupta and Roper, 1994). However, alternative farmers in Australia may make greater use of tillage (and fallow) than conventional farmers, as herbicides are not an option for weed control.

The non-intensive nature of much Australian agriculture may result in slight differences in management not leading to detectable differences in the soil biota. For instance, Fettell *et al.* (1994) found that in low rainfall environments where crop stubble production is relatively low and where tillage operations are rarely intensive, the choice of stubble management and tillage methods did not have a large or long-term impact on the soil biota.

However, a major difference between alternative and conventional farms in Australia involves the use of fertilisers, particularly phosphatic fertilisers. The use of soluble P fertilisers on conventional farms will dramatically reduce the level of colonisation by VAM fungi, which could potentially have a negative effects in a number of areas including maintenance of soil structure (Gatehouse, 1995). There is little information available on other effects of fertilisers on soil organisms, however it has been suggested that all significant effects will be mediated through the influence of the fertilisers on plant growth (Rovira, 1994). The effect of soil organisms other than VAM fungi on plant nutrient uptake is another area requiring further investigation

Biocides have been shown to effect the functioning of various groups of soil organisms (Fraser, 1994; Gupta, 1994), with insecticides, fungicides and fumigants generally having more severe impacts than herbicides. Studies that report enhanced biological activity in soils on alternative farms generally do not directly attribute this to the use of biocides in the conventional system. Although, presumably the use of biocides in conventional intensive horticulture or orchard operations could result in differences in the soil biota in comparison to alternatively managed operations. However, in extensive cropping or livestock operations in Australia the use of these chemicals is minimal and may have no significant impact on the soil biota, particularly in the long-term.

While the addition of organic matter or other management practices on alternative farms may lead to increased soil biological activity, there is little known about the effects of this on the functioning of the entire system. For instance, it is not known whether enhanced biological activity results in a system being more resilient to disturbances and stresses, such as drought.

6. Conclusions

Although there has been little research it appears that enhanced soil biological activity, relative to conventional systems, may be a feature of alternative farms in Australia. In particular, this is likely to be the case in more intensive operations, such as horticulture and orchards, where large inputs of organic matter may occur under alternative management.. However, when the biomass or composition of the soil biota differs from conventional farms, there is a lack of information about the consequences of this for the functioning of the entire system.

It is likely that important differences, as yet undetected, may exist between alternative and conventional systems in the occurrence or activities of particular groups or species of soil organisms. These differences may not be reflected in broad measures of

microbial activity, yet may result in different pathways being present for processes such as disease suppression and plant nutrient uptake.

7. References

A.Q.I.S. 1992. National standard for organic and bio-dynamic produce Organic Produce Advisory Committee, A.Q.I.S., Canberra.

Abbott, L. K. and Robson, A. D. 1994. The impact of agricultural practices on mycorrhizal fungi, Soil Biota: Management in Sustainable Farming Systems, CSIRO, Adelaide, Australia.

Baker, G. H., Carter, P. J., Barrett, V. J., Kilpin, G. P., Buckerfield, J. C. and Dalby, P. R. 1994. The introduction and management of earthworms to improve soil structure and fertility in south-eastern Australia, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Bokhorst, J. G. 1989. 'The organic farm at Nagele', *In* Development of farming systems. (Eds. Zadoks), Pudoc, Wageningen.

Bolan, N.S., White, R.E, and Hedley, M.J. 1990. A review of the use of phosphate rocks as fertilisers for direct application in Australia and New Zealand, Aust. J. Exp. Agric., **30**: 297-313.

Buckerfield, J. C. and Auhl, L. H. 1994. Earthworms as indicators of sustainable production in intensive cereal cropping, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Coleman, D. C., Andrews, R., Ellis, J. E. and Singh, J. S. 1976. Energy flow and partitioning in selected man-managed and natural ecosystems, Agro-Ecosystems, **3**: 45-54.

Coventry, D. R., Hirth, J. R. and Reeves, T. G. 1985. Development of populations of *Rhizobium trifolii* and nodulation of subterranean clover following the cropping phase in crop-pasture rotations in southeastern Australia, Soil Biol. Biochem., **17**: 17-22.

Daamen, R. A., Wijnands, F. G. and van der Vliet, G. 1989. Epidemics of diseases and pests of winter wheat at different levels of agrochemical input, J. Phytopath., **125**: 305-319.

Dann, P. R., Derrick, J. W., Dumaresq, D. C. and Ryan, M. H. 1996. The response of organic and conventionally grown wheat to superphosphate and reactive rock phosphate, Aust. J. Exp. Agric., **36**: 71-78.

Derrick, J. W. 1996. A comparison of agroecosystems: organic and conventional broadacre farming in south east Australia, Australian National University, Canberra.

Elmholt, S. and Kjøller, A. 1989. Comparison of the occurrence of the saprophytic soil fungi in two differently cultivated field surveys, Biol. Agric. Hort., **6**: 229-239.

Fettell, N. A., Carpenter, D. J., Kidston, J. and Gupta, V. V. S. R. 1994. Organic matter, microbial biomass and respiration after 13 years of stubble retention and zero-tillage in western NSW, Soil Biota: Management in Sustainable Farming Systems, CSIRO, Adelaide.

Fraser, P. M. 1994. The impact of soil and crop management practices on soil macrofauna, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Gatehouse, R. 1995. Mycorrhizae, soil management and soil structure in a neighbouring organic and conventional dryland wheat farm at Ardlethan, NSW, Australian National University, Canberra.

Gupta, V. V. S. R. 1994. The impact of soil and crop management practices on the dynamics of soil microfauna and mesofauna, Soil Biota: management in sustainable farming systems, CSIRO, Adelaide.

Gupta, V. V. S. R. and Roper, M. M. 1994. Effect of stubble management on the functional groups of soils microorganisms, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Hoitink, H. A. J. and Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts, Ann. Rev. Phytopath., **24**: 63-114.

Janke, R. R., Mt Pleasant, J., Peters, S. E. and Böhlke, M. 1991. Long-term, low-input cropping systems research, Sustainable Agriculture Research and Education in the Field, National Academy Press, Washington DC.

La Rooj, M. 1989. 'Soil fertility', *In* Biodynamics: new directions for farming and gardening in New Zealand. Random House, Auckland, pp. 18-24.

Ledgard, S. F. and Steele, K. W. 1992. 'Biological nitrogen fixation in mixed legume/grass pastures', *In* Biological Nitrogen Fixation for Sustainable Agriculture. (Eds. Ladha, J. K., T. George and B. B. Bohlool), Kluwer, Dordrecht, pp. 137-153.

Leeper, G. W. and Uren, N. C. 1993. Soil Science: An Introduction Melbourne University Press, Melbourne.

Lengnick, L. L. and King, L. D. 1986. Comparison of the phosphorus status of soils managed organically and conventionally, Am. J. Alternative Agric., **1**: 108-114.

Lopez-Real, J. M. 1985. 'Sustainable agriculture: the microbial potential- the microbiologists challenge', *In* The Role of Micro-Organisms in a Sustainable Agriculture. (Eds. Lopez-Real, J. M. and R. D. Hodges), Academic, Berkhamstead.

Macgregor, A. N. 1994. Beneficial soil biota in organic and alternative farming systems, Soil Biota: Management in Sustainable Farming Systems, CSIRO, Adelaide.

National Research Council 1989. Alternative Agriculture National Academy Press, Washington, D.C.

Neate, S. M. 1994. Soil and crop management practices that affect root diseases of crop plants, Soil Biota: Management in Sustainable Farming Systems, CSIRO, Adelaide.

Pankhurst, C. E., Hawke, B. G., McDonald, H. J., Kirkby, C. A., Buckerfield, J. C., Michelsen, P., O'Brien, K. A., Gupta, V. V. S. R. and Doube, B. M. 1995. Evaluation of soil biological properties as potential bioindicators of soil health, Aust. J. Exp. Agric., **35**: 1015-1028.

Penfold, C. M., Miyan, M. S., Reeves, T. G. and Grierson, I. T. 1995. Biological farming for sustainable agricultural production, Aust. J. Exp. Agric., **35**: 849-856.

Price, P. W. 1988. An overview of organismal interactions in ecosystems in evolutionary and ecological time, Agric. Ecos. Environ., **24**: 369-377.

Reganold, J. P. 1988. Comparison of soil properties as influenced by organic and conventional farming systems, Am. J. Alternative Agric., **3**: 144-155.

Robertson, F. A. and Morgan, W. C. 1996. Effects of management history and legume green manure on soil microorganisms under 'organic' vegetable production, Aust. J. Soil Res., **34**: 427-440.

Roget, D. K. 1995. Decline in root rot (*Rhizoctonia solani* AG-8) in wheat in a tillage and rotation experiment at Avon, South Australia, Aust. J. Exp. Agric., **35**: 1009-1014.

Rovira, A. D. 1994. The effect of farming practices on the soil biota, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Ryan, M. H. 1992. Soil fungi on two adjacent wheat farms: comparative effects of organic and conventional management, Australian National University, Canberra.

Ryan, M. H. and Ash, J. E. 1996. Colonisation of wheat in southern NSW by VA-mycorrhizal (VAM) fungi is significantly reduced by drought, Aust. J. Exp. Agric., **36**: 563-569.

Ryan, M. H., Chilvers, G. A. and Dumaresq, D. C. 1994. Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour, Plant Soil, **160**: 33-40.

Sattelmacher, B., Reinhard, S. and Pomikalko, A. 1991. Differences in mycorrhizal colonisation of rye (*Secale cereale* L.) grown in conventional or organic (biological-dynamic) farming systems, J. Agron. Crop Sci., **167**: 350-355.

Schnürer, J., Clarholm, M. and Rosswall, T. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents, Soil Biol. Biochem., **17**: 611-618.

Sinnamon, L. 1996. Why grow food organically?, WellBeing Magazine, **63**: 6-9.

Sivapalan, A., Morgan, W. C. and Franz, P. R. 1993. Monitoring populations of soil microorganisms during a conversion from a conventional to an organic system of vegetable growing, Biol. Agric. Hort., **10**: 9-27.

Small, D., McDonald, J. and Wales, B. 1994. Alternative Farming Practices Applicable to the Dairy Industry Department of Agriculture, Victoria & The Dairy Research and Development Corporation, Kyabram.

Springett, J. A. 1994. The biodiversity of the soil fauna under two types of pasture management in New Zealand, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Springett, J. A. and Gray, R. A. J. 1994. The distribution of pasture roots and earthworm burrows in the soil profiles of a conventional and an organic dairy farm, Soil Biota: Management in sustainable farming systems, CSIRO, Adelaide.

Thompson, J. P. 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake, Soil Biol. Biochem., **26**: 1133-1143.

Thompson, J. P. and Wildermuth, G. B. 1989. Colonisation of crop and pasture species with vesicular-arbuscular mycorrhizal fungi and infection by *Bipolaris sorokiniana*, Can. J. Bot., **67**: 687-693.

Tisdall, J. M. and Oades, J. M. 1979. Stabilization of soil aggregates by the root systems of ryegrass, Aust. J. Soil Res., **17**: 429-441.

van Bruggen, A. H. C. 1995. Plant disease severity in high-input compared to reduced-input and organic farming systems, Plant Disease, **79**: 976-984.

Werner, M. R. and Dindal, D. L. 1990. Effects of conversion to organic agricultural practices on soil biota, Amer. J. Alternative Agric., **5**: 24-32.

Werner, M. R., Kluson, R. A. and Gliessman, S. R. 1990. Colonisation of strawberry roots by VA mycorrhizal fungi in agroecosystems under conventional and transitional organic management, Biol. Agric. Hort., **7**: 139-151.

Witter, E., Mårtensson, A. M. and Garcia, F. V. 1993. Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilisers and organic amendments, Soil Biol. Biochem., **25**: 659-669.

Workneh, F. and van Bruggen, A. H. C. 1994. Microbial density, composition, and diversity in organically and conventionally managed rhizosphere soil in relation to suppression of corky root of tomatoes, Appl. Soil Ecol., **1**: 219-230.

Wynen, E. 1992. Conversion to organic agriculture in Australia: problems and possibilities in the cereal-livestock industry The National Association for Sustainable Agriculture Australia Ltd, Sydney.